

Secretariaat  
dr. F.H.M. Borgsteede  
Centraal Diergeneeskundig Instituut  
Postbus 65  
8200 AB Lelystad

6975/88

Dr. A. de Geus  
Editor T.G.M.  
c/o K.I.T.  
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Geachte collega de Geus,

Gewoontegetrouw zend ik U na onze najaarsvergadering de abstracts van de aldaar gehouden lezingen en gepresenteerde posters. Een aantal van onze leden heeft reeds een poster gepresenteerd tijdens het grote congres in september in de RAI en heeft dezelfde poster gebruikt voor onze najaarsvergadering. Niet al onze leden waren immers in Amsterdam. Wij hebben gemeend de abstracts van deze posters niet voor plaatsing aan te bieden, omdat de abstracts reeds in het abstractsboek van het congres zijn opgenomen.

Graag wil ik van deze brief ook gebruik maken om onze tevredenheid te betuigen en U dank te zeggen voor de snelle plaatsing van de abstracts van de afgelopen twee vergaderingen. De achterstand die er bestond, lijkt nu weggewerkt en hopelijk kunt U het tempo bijhouden.

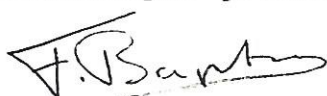
Zoudt U de bijgesloten abstracts willen plaatsen onder de kop:

NETHERLAND      NETHERLANDS SOCIETY FOR PARASITOLOGY

Autumn meeting Lelystad, 11 November 1988

De volgorde van de abstracts is met potlood rechtsboven aangegeven.

Met vriendelijke groet,



Dr. F.H.M. Borgsteede, secretaris N.V.P.

Trop. geogr. Med.

TRANSCRIPTION AND RNA POLYMERASES: A UNIQUE SITUATION IN KINETOPLASTIDA

A.W.C.A. CORNELISSEN, R. EVERS, E. GRONDAL, A. HAMMER, W. JESS AND J. KÖCK

Max-Planck-Institut für Biologie, Molecular Parasitology Unit, Spemannstrasse 34, 7400 Tübingen, FRG

Transcription in eukaryotes is regulated by three classes of DNA-dependent RNA polymerases. These are named I, II, and III according to their order of elution during ion-exchange chromatography and on basis of their differential alpha-amanitin sensitivity (1). Transcription of protein coding genes in eukaryotes is performed by RNA Polymerase II, which activity is strongly inhibited by alpha-amanitin. However, the transcription of the VSG genes of the African trypanosome *Trypanosoma brucei* is, despite the protein coding nature of these genes, resistant to alpha-amanitin. The eukaryotic RNA polymerase resistant to alpha-amanitin is RNA polymerase I (1). Hence, this polymerase might transcribe the VSG genes. Alternatively, VSG genes might be transcribed by a modified, alpha-amanitin-resistant RNA polymerase II. To decide between these two possibilities, we have started to characterise the RNA polymerases of trypanosomes. Our recent work (2-3) can be summarized as follows:

1. The genes encoding the largest subunit of RNA polymerase I, II, and III have been identified, cloned and sequenced. In parallel, we also separated the trypanosomal RNA polymerases by DEAE-Sephadex chromatography. The elution pattern and the characteristics of the individual peaks were essentially as described for eukaryotic RNA polymerases I, II, and III. Taken together, these experiments suggest that *T.brucei* possesses the normal three classes of eukaryotic RNA polymerases.

2. An additional locus encoding a RNA polymerase II-like sequence was identified in *T.brucei* and related kinetoplastid species. The presence of two genes suggest that a fourth RNA polymerase - a modified alpha-amanitin resistant RNA polymerase II - could be formed. However, further experiments are needed to resolve this matter conclusively.

A summary of these results will be presented.

This work was supported by the Bundesministerium für Forschung und Technologie grant 0318885 and by a research award of the Erna and Victor Hasselblad Foundation.

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# CEREBRAL MALARIA: A COMPARISON OF HUMAN AND RODENT RESEARCH.

J.H.A.J. CURFS and W.M.C. ELING.

Department of Celbiology and Histology, Catholic University of Nijmegen,  
Nijmegen, The Netherlands.

The most dangerous complication of malaria is cerebral malaria. In humans it may develop after infection with Plasmodium falciparum. About 20-40% of the patients that are admitted to hospital with cerebral malaria die despite treatment. In Africa predominantly young children are affected, but in Asia, for example in Viet Nam, it affects adults too. Although cerebral malaria has been studied for nearly a century, little is known about its pathogenesis and the cause of coma, despite extensive descriptive literature on the pathology itself. Therefore, it should be extremely helpful if animal models resembling the human situation could be developed.

There are several rodent models, including mouse and rat, with cerebral malaria, but it remains to be seen whether these resemble the human situation in its pathogenesis. With respect to the pathology itself there are many similarities between human and rodent cerebral malaria, however there are striking differences too. In human cerebral malaria sequestration of parasitized erythrocytes is believed to play an important role in the development of coma. In rodent cerebral malaria, the pathogenesis is believed to arise from an immunopathological T-cell reaction that is triggered by parasitic antigen. Several immunosuppressive measures can prevent the development of this immunopathological reaction (such as splenectomy, irradiation of the spleen or a timely treatment with anti T-cell serum or corticosteroids). Apart from immunosuppressive measures, timely treatment with antibodies present in mouse immune-serum can also prevent the development of cerebral malaria, indicating that protection is possible when an adequate immune response is developed [1].

Damage to the endothelial lining, characteristic in rodent cerebral malaria, might be due to the action of cytokines, amongst which TNF may be an important one. Behavioural changes in endothelial cells (i.e. phagocytosis) may cause endothelial damage too, resulting in haemorrhages in the brain followed by death of the animal.

We have shown in a Plasmodium berghei-mouse model that it is possible to immunize / vaccinate against cerebral malaria with live / dead parasitized erythrocytes. Currently we are trying to determine that part of the parasite that is responsible for the triggering of the immunopathological reaction and to use it in vaccination experiments.

In Plasmodium falciparum cerebral malaria, it is not known whether immunopathological reactions play an important role. Some believe that acidosis, as result of vascular obstruction, leads to the observed pathology [2].

Further observations of both the human and rodent cerebral malaria must be made to determine whether the results of murine cerebral malaria apply to the human situation.

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# IN VITRO CULTURE OF VIVAX-TYPE MALARIA PARASITES.

B. MONS, E.G. BOORSMA, C.J. JANSE.

Laboratory for Parasitology, University of Leiden,  
The Netherlands.

Of the vivax-type malaria parasites, the rodent parasite Plasmodium berghei is most widely used as a model system to study the cell biological, molecular biological and immunological aspects of the life cycle of this type of plasmodia.

One of the most important prerequisites for such studies is a reproducible method for the in vitro cultivation of the parasite.

A culture method for the human parasite P. vivax has recently been described but is at present only reproducible for short-term experiments in vitro using reticulocytes from human or non-human primate sources [1,2]. P. berghei infects a number of standard laboratory rodents, and both the exoerythrocytic and the erythrocytic stages can be reproducibly cultured in vitro [3,4]

A significant drawback of vivax-type malaria parasites in general is the restriction in the capability to invade mature red blood cells (rbc), usually described as "reticulocyte preference".

This hampers large scale cultivation of the parasites.

Recently a report has been published which described a culture system in which P. berghei did invade mature rbc from a variety of hosts, and could be cultured for prolonged periods at 27 C with extremely high multiplication rates [5,6].

We reconsidered the host cell specificity of two major strains of P. berghei (ANKA and K173) under different in vitro conditions.

The development in vitro of parasites from both strains was severely inhibited under the culture conditions as described by Ramaiya et al. [5,6]. Merozoites of both strains showed a strict preference for reticulocytes. Red blood cells from rats, mice and hamsters were readily invaded by merozoites from both strains. Erythrocytes from rabbits and guinea pigs were resistant to invasion by P. berghei.

It is concluded that host cell specificity technically limits the possibilities for mass cultivation of P. berghei and P. vivax. The validity of the recent publications [5,6] describing alternative conditions is questioned.

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**Intervet**

4

**NATURAL ANTIBODIES AND THE SIGNIFICANCE OF SELF-RECOGNITION IN PARTICULAR IN MALARIA INFECTIONS**SCHETTERS, T.\*<sup>Δ</sup>, BROWN, K.N.<sup>Δ</sup>, JARRA, W.<sup>Δ</sup> AND ELING, W.\*

\* *Department of Cytology and Histology, University of Nijmegen, Nijmegen, The Netherlands.*

Δ *Department of Parasitology, National Institute for Medical Research, London, U.K..*

Recognition of non-self is based on self-recognition. Natural antibodies (physiological antibodies) have antigen binding sites which recognize self-structures, and thus provide the template for such self-recognition. They not only play a part in classical immune reactivity, but also function in other physiological processes. Immunoglobulins reactive with erythrocyte surfaces have been found by different groups, using different techniques. It has been suggested that they play a role in erythrocyte physiology. An eluate prepared from normal mouse erythrocytes showed selective elution of IgM and IgG3 as compared to the amount in plasma. The eluted (physiological) antibody can reversely bind to erythrocytes. This interaction can be partially blocked by the addition of phosphocholinechloride (PCC), but not cholinebromide or para-aminophenylcholine. Furthermore PCC does not interfere with binding of anti-TNP antibodies to TNP, indicating the specificity of the interaction. Polyclonal activation of lymphocytes, as has been described for malaria infection, increases the physiological antibody level. High levels of physiological erythrophilic antibodies might interfere with merozoite attachment and penetration of erythrocytes. Using in vitro techniques interference of physiological antibodies with penetration of erythrocytes by *Plasmodium falciparum* could not be demonstrated. Transfer of sera from *Plasmodium berghei* infected or immune mice showed that erythrocyte protective activity of these sera was correlated with the amount of IgG3 in the serum samples. Eluates prepared from normal erythrocytes protect erythrocytes from complement mediated lysis (in vitro).

It is suggested that erythrophilic antibody produced in higher amounts in *Plasmodium berghei* infected and immune mice, is protective to the host, by inhibiting erythrocyte destruction.



# DETECTION OF THE SCHISTOSOME CIRCULATING ANODIC ANTIGEN (CAA) FOR IMMUNODIAGNOSIS OF SCHISTOSOMIASIS

N. DE JONGE and A. M. DEELDER.

*Laboratory of Parasitology, Medical Faculty, University of Leiden, The Netherlands.*

The diagnosis of schistosomiasis is based on indirect methods: usually parasitological methods for the detection of the parasite eggs in the excreta, or serological methods for the demonstration of patient's antibodies directed against parasite material are applied. These methods, however, are not without drawbacks. There is a large day-to-day fluctuation in the egg excretion, antibody titres show a very poor correlation with the worm burden and antibody levels may remain elevated for months after chemotherapy. The more direct detection of schistosome circulating antigens would provide a means to overcome these problems.

One of the major schistosome circulating antigens is the Circulating Anodic Antigen (CAA). This is a proteoglycan with a molecular weight of approximately 70,000 dalton. It is released by the worms into the host's bloodstream by regularly regurgitating undigested food components.

We developed a monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) for the detection of CAA. This assay appeared to be entirely specific and very sensitive: the lower detection limit is about 10 pg CAA per ml serum. CAA was demonstrated in the serum of patients infected with *S. haematobium*, *S. intercalatum*, *S. japonicum* and *S. mansoni* and in the urine of patients with *S. haematobium* and *S. mansoni* infections. A strong correlation was found between CAA-levels in serum and the worm burden, as measured by the egg output. CAA levels showed less day-to-day fluctuation than egg excretion. The presence of CAA indicates an active infection: after successful chemotherapy, serum-CAA levels drop to less than 10 % of the pre-treatment values within two weeks.

This study received financial support from the Research and Development Programme 'Science and Technology for Development' of the European Community and the WHO/UNDP/World Bank Special Programme for Research and Training in Tropical Diseases.

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6

SKIN AND LEATHER DAMAGE CAUSED BY ECTOPARASITES OF CATTLE  
W.M.L. HENDRIKX\*, E. GRUYS\*\* and H.H.A. PELCKMANS\*\*\*

\*Department of Infectious Diseases and Immunology and \*\*Pathology,  
Veterinary Faculty, University of Utrecht, \*\*\*Institute for Leather  
and Shoes, TNO, Waalwijk, The Netherlands

Damage to hides and skins of cattle can occur intra vitam or post mortem. This damage has a biological or mechanical origin. Infectious agents like viruses, bacteria, moulds, mites, ticks and insects interact with the host, causing disease. This interaction can alter the normal skin. Healing of altered skin is not always complete and scars are left. The latter result in leather damage, when hides and skins are tanned. Wounds of iatrogenic or mechanical origin, improper flaying of hides obviously will lead to mechanical leather damage.

Examples of leather damage were shown with emphasis on the effects of ectoparasites of cattle. In the Netherlands Hypoderma bovis is practically eradicated, however, imported cattle may be infected. Ticks and the hair follicle mite Demodex occur on cattle but disease or damage to leather is practically not seen. Surveys of cattle herds on the prevalence of mange mites and lice should be propagated to establish the role of these parasites on hide and leather quality. A survey on 90 dairy cattle and 10 mastbull farms in the province of Limburg demonstrated, that on 70% of the farms infections with ectoparasites occurred. Chorioptes bovis and Damalinia bovis were found on 40-50% of the farms, sucking lice occurred on 10% of the dairy farms. Psoroptes ovis was incidentally found and Sarcoptes scabiei was not found. Clinical observation showed that trichophyty occurred on 31% of the dairy farms and on 70 % of the mastbull farms. Especially Psoroptes ovis, Sarcoptes scabiei and Trichophyton verrucosum are hazards in regard to leather quality [1, 2].

We investigated the correlation between ectoparasitic infections and skin and leather damage in 20 selected calf skins. Fresh skin halves were tanned and leather damage was recorded. The counter halves showed mixed infections of Damalinia with Linognathus (5x) respectively with Chorioptes (1x) or mono-infections with the same species (4x, 1x respectively 2x). These 13 skins showed inflammatory reactions with damage to the basal membrane and the leather halves showed light spot/light fleck leather damage. On 3 skins no ectoparasites nor histological changes were found and the 3 parallel leather halves were unaffected. In 4 skins no ectoparasites were found, but histological changes correlated with damage on the leather halves [3].

This study suggests a correlation between the presence of ectoparasites, damage to the basal membrane and light spot/light fleck leather damage. Further study is necessary to exclude other possible causes.

These studies were supported by the Dutch Commission on Hide Quality respectively by the Organisation of Live Stock and Meat (PVV).



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7)

SNAIL-SCHISTOSOME INTERACTIONS: HAEMOCYTE-MEDIATED KILLING AND  
PARASITE STRATEGIES TO CIRCUMVENT IT

W.P.W. VAN DER KNAAP, E.A. MEULEMAN, E.C. VAN DEUTEKOM-MULDER,  
R.I. AMEN, C.M. ADEMA and <sup>1</sup>T. SMINIA

*Laboratory of Medical Parasitology and <sup>1</sup>Department of Histology,  
Faculty of Medicine, Free University, Amsterdam, The Netherlands*

After injection or penetration in *Lymnaea stagnalis*, miracidia of compatible *Trichobilharzia ocellata* as well as those of incompatible *Schistosoma mansoni* transform into mother sporocysts. The epidermal plates and loose cilia they shed in the haemolymph of the snail are phagocytosed by the circulating effector cells of the snail's internal defence system, the haemocytes. In the incompatible combination the sporocyst is then encapsulated by haemocytes, its newly-formed tegument is breached and its remnants are phagocytosed. During encapsulation the haemocytes have been shown *in vitro* to release reactive forms of oxygen and the enzyme peroxydase on to the sporocyst tegument, which suggests a killing mechanism similar to the one underlying the killing of schistosomula by mammalian leucocytes. Haemocyte subpopulations involved in the above processes have been characterized by electron microscopy and with monoclonal antibodies and lectins as probes.

In the compatible combination the snail is unable to recognize or destroy the sporocyst and the parasite develops normally. With lectin binding studies and immunocytochemistry, similarity was demonstrated in antigenic surface determinants on miracidia and mother sporocysts on the one hand and snail tissue on the other. This antigenic similarity also holds for cercariae after but not before their escape from the daughter sporocyst in the haemolymph of the snail. Moreover, mother sporocysts have been shown to produce substances which modulate haemocyte activity. Thus, molecular mimicry and masking as well as immunosuppression may be used by the parasite as strategies to circumvent immunorecognition and killing. These strategies may be more successful in juvenile (highly susceptible) snails than in adult (less susceptible) ones, because the juvenile defence system is not yet mature. This immaturity was demonstrated by comparing humoral and cellular defence activities in both age groups.



ABSTRACT POSTER NVP najaarsvergadering 1988 (NVPOS88.ABS)

THE RATE OF OXYGEN RADICAL GENERATION BY HEMOCYTES OF THE SNAIL LYMNAEA STAGNALIS IN RELATION TO THE KINETICS OF PHAGOCYTOSIS.

C.M. ADEMA, W.P.W. VAN DER KNAAP, T. SMINIA<sup>1</sup>.

Laboratory of Medical Parasitology, Department of Histology<sup>1</sup>, Faculty of Medicine, Free University Amsterdam.

Understanding of the immune mechanisms active in parasite/snail interactions will lead to better insight into schistosome/snail compatibility and thus into transmission of schistosomiasis.

During interaction with foreign particles, snail blood cells (hemocytes) generate potentially cytotoxic oxygen radicals (<sup>1,2</sup>). In our study of the cytotoxicity mechanism in hemocytes of the pond snail Lymnaea stagnalis, intermediate host of Trichobilharzia ocellata, we compared the kinetics of oxygen metabolite production in relation to the kinetics of phagocytic activity using zymosan stimulated hemocyte monolayers.

Until number of phagocytosing hemocytes and number of particles per cell had reached a maximum, oxygen radicals (superoxide and hydrogen peroxide) were detected mainly extracellularly. Thereafter intracellular generation of oxygen radicals (singlet oxygen) increased whereas extracellular production decreased.

Adequate stimulation of hemocytes is essential for detectable production of oxygen radicals. High phagocytic activity, yielding high oxygen radical production, was obtained by establishing hemocyte/particle contact prior to the initiation of phagocytosis by a temperatureshift from 0°C to 20°C. Since triggering and regulation follow similar patterns, the mechanism of oxygen radical generation in snail hemocytes may be analogous to the mechanism of oxygen radical generation in mammalian leucocytes, known as respiratory burst. Further study is in progress.

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GENERATION OF T-CELL LINES SPECIFIC FOR CRUDE TRICHINELLA SPIRALIS MUSCLE LARVE-DERIVED ANTIGENS

C.M. MINK<sup>1</sup>, B. VAN DER PLAS<sup>2</sup>, J. BUIJS<sup>2</sup>, W.E. BERNADINA<sup>1</sup> and E.J. RUITENBERG<sup>1,2</sup>

<sup>1</sup>Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, University of Utrecht, The Netherlands

<sup>2</sup>Department of Parasitology, RIVM, Bilthoven, The Netherlands

T cells are involved in the development of both protective immunity and pathology following infections with *Trichinella spiralis*.

Preparation of T-cell lines/clones which display these effects in vivo will be helpful to assess (a) the roles of T cells in regulating the host response, and (b) the *T. spiralis* epitopes triggering the generation of immunity/pathology inducing T cells. We employed the following scheme to generate mouse and rat T-cell lines.

As starting lymphocyte populations were used mesenteric lymph node cells from Balb/c mice taken 7 days post infection (p.i.), and from PVG rats taken 12 and 28 days p.i. Restimulations with antigen were carried out every 7-10 days depending on cell growth. Three days after each restimulation IL-2 was added to the culture. Irradiated spleen cells were used as antigen presenting cells.

Rat T-cell lines were stable in culture for up to 8 weeks, retaining antigen specificity for 6 weeks. After 6 weeks in culture increased cell proliferation was observed, albeit no antigen specificity was detected by LST.

Mouse T-cell lines were stable in culture for up to 9 weeks, retaining the antigen specificity. The in vivo functions of these cultured T cells are, as yet, undefined. FACS analysis of mouse T cells, cultured for 6 weeks, demonstrated that most T cells were L3T4-positive, indicating helper functions.

Data concerning our approach will be presented.



ANTIBODIES AGAINST A 26 KD PARASITE SURFACE ANTIGEN INHIBITS  
PROLIFERATION OF THE PARASITE (PLASMODIUM BERGHEI).

C.C. HERMSEN, I. SCHOLTEN, C. VAN DE LEST AND W.M.C. ELING.  
Department of Celbiology and Histology, University Nijmegen,  
The Netherlands.

In the analysis of the response of hosts immune against Plasmodium species it is important to identify immunogenic molecules of parasites and parasitized erythrocytes. Such molecules can be used to monitor immune effector functions and might be of importance for the development of effective vaccines.

A widely used strategy is the search for antibodies in serum from immune individuals that react with parasite surface antigens or parasite derived antigens of parasitized erythrocytes.

Another strategy is to use proteins of parasitized erythrocytes which are obtained by PAGE and electroelution. Animals are immunized with these proteins and protection is determined. Patarroyo et al. [1] made synthetic peptides corresponding to fragments of these proteins which were successfully used in monkey immunization trials. These synthetic peptides vaccines were also considered successful in a human trial.

We have used surface elution of antibodies out of immune serum to detect surface antigens of parasitized erythrocytes of Plasmodium berghei. A parasite derived molecule with a relative molecular mass of 26 kD was recognized. With the use of heterologous antibodies against 26 kD we found immunofluorescence of the parasitized erythrocytes and the immunogold labelling technique with the anti 26 kD antibodies showed that the surface of the infected erythrocyte was labeled. The heterologous antiserum was protective in-vivo, in that it blocked infection when low numbers of living parasites were used to infect the animals. Mice which were vaccinated with the 26 kD polypeptide exhibited a decreased proliferation of the parasite.

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# MALARIA AND ANTI-SPOROZOITE ANTIBODIES DURING PREGNANCY IN A WEST-AFRICAN TOWN.

Bekkers R., Verhave J.P., v.d.Meulen F., Cot M.  
Katholic University of Nymegen, Holland and Antenne ORSTOM, Bobo  
Dioulasso, Burkina Faso.

During april-oktober 1987, 482 pregnant women in Banfora, Burkina Faso, were recruited and followed parasitologically and serologically during their pregnancies until delivery. Each month and at delivery, blood was examined.

During april-december 87 the blood of a group of 185 non-pregnant women was also examined.

Of the 482 women, 81 (17%) was primigeste. 76,3% was older than 20 years. Among the primigestes only 14.8% was older than 20.

276 of the women got profylaxis of 300 mg cloroquine-base weekly. All blood samples were examined by microscope on a thin and a thick smear and serologically by ELISA, using a recombinant circumsporozoite antigen. Placentae were examined for the presence of parasites.

The goal was to get information about the role of anti-sporozoite antibodies in the loss of immunity which is generally observed during pregnancy, particularly in primiparous women.

Also in our study we find primiparous woman more susceptible to parasitemia and placenta infestation with parasites.

Both, primi's and multi's, showed a significantly higher ab-titer at 5 months of pregnancy compared with their titers at delivery, while the titers at 5 months didnot differ from those in the control-group. This indicates a loss of antibodies in the second half of pregnancy which may partially be a cause for the increased susceptibility.

The ab-titer did show a significant difference between primi's and multi's, but we could show that this difference was almost totally due to the difference in age; instead of being parity-dependend ab's rather seem to be age-dependend.

We were not able to show that this difference in ab-titers between primi's and multi's was the cause for differences in malaria susceptibility between these groups. In fact, we could show that among the women with a strongly positive ab-titer ( $>1/160$ ), primi's had a much bigger share in the group which went through one or more parasitemias during pregnancy, compared with the share in the group with no parasitemia during pregnancy.

These findings raised the question if their was any effect at all of these ab's on parasitemia and/or placenta infestation.

In the group which got prophylaxis we couldnot show an effect of the ab's on parasitemia nor on placenta-infestation but within the group without prophylaxis, their was a slight difference in malaria susceptibility between the group negative for ab's and the group which was strongly positive for both parasitemia and placenta infestation. This effect was stronger with the titer at 5 months compared with the titer at delivery.

This difference indicates that a part of those parasitemias is caused by recrudescence rather than re-infection. The reason for these recrudescences to occur is that, similar to the loss of anti-sporozoite antibodies, there may be a loss of other plasmodial antibodies which attack the blood stages of malaria.



AN INVESTIGATION OF THE ENDOPARASITIC HELMINTH FAUNA OF ROE DEER  
(CAPREOLUS CAPREOLUS L.) IN THE NETHERLANDS

F.H.M.BORGSTEEDE<sup>1</sup>, J.JANSEN<sup>2</sup>, H.P.M.VAN NISPEN TOT PANNERDEN<sup>2</sup>, N.NOORMAN<sup>1</sup>,  
W.P.J.VAN DER BURG<sup>1</sup>, J.POUTSMA<sup>3</sup> and J.F.KOTTER<sup>4</sup>

<sup>1</sup>Central Veterinary Institute, Lelystad, The Netherlands

<sup>2</sup>Institute of Veterinary Parasitology, University of Utrecht, Utrecht, The Netherlands

<sup>3</sup>Lokveenweg 38, Haren, The Netherlands

<sup>4</sup>Vaart OZ 5, Warfhuizen, The Netherlands

Eighty eight roe deer from four regions were investigated from May 1986 until May, 1987 for the presence of helminth endoparasites. All roe deer were found to be infected with at least one worm species. The following species were found in the abomasum : *Spiculopteragia boehmi* (79 % of the roe deer positive), *Ostertagia leptospicularis* (76 %), *Skryabinagia kolchida* (73 %), *Trichostrongylus axei* (49 %), *Rinadia mathevossiani* (16 %), *O.ostertagi* (16 %), *Haemonchus contortus* (3%) and *O.circumcincta* (1%). *Capillaria bilobata* was found in the abomasum/small intestine of 6 % of the roe deer. *T.capricola* (49 %), *Nematodirus europaeus* (31 %) and *Bunostomum trigonocephalum* (2% ) were present in the small intestine. *Trichuris ovis* (49 %), *Oesophagostomum venulosum* (13 %) and *Chabertia ovina* (2 %) were present in the large intestine and/or caecum. *Dictyocaulus noerneri* was found in 6 % of the roe deer lungs. No helminths were found in the rumen and the liver. The highest gastro-intestinal worm burden was 30.600. Differences in worm numbers and in species composition were demonstrated between regions and between age groups. *C.bilobata*, *B.trigonocephalum* and *Ch.ovina* have not been described earlier from roe deer from The Netherlands.

**Immunoblotting technique in diagnosis of Toxoplasma gondii infections in heart transplant recipients. Development of technique and application.**

J.J. Verweij, J.F. Sluiters and J.P. Rotmans

Dept. Clin. Microbiology, Erasmus University, Rotterdam and Lab. for Parasitology, State University of Leiden, Leiden, The Netherlands

Several conditions in the technique of SDS-polyacrylamide gel electrophoresis and immunoblotting were compared in the detection of IgG and IgM antibodies to Toxoplasma gondii in sera of experimentally infected rabbits. Miniblotting gave similar results as the macroblot system and was used throughout the further study. Band patterns of blots differed between antigenic preparations of Toxoplasma Deelen strain and RH strain when assayed with the same antiserum. More consistent results in detection of antibodies to low molecular weight range (below 30 kiloDalton) were obtained with immobilon polyvinylidene difluoride transfer membrane (PVDF; Millipore) than with nitrocellulose membrane of 0.45, 0.2 or 0.1  $\mu$ m pore width (Schleicher and Schüll). Application of immunoblotting to subsequently taken sera of heart transplant recipients showed the appearance of antibodies to 6 kD antigen in IgM blot in primary Toxoplasma gondii infections as most conspicuous. In reactivations of latent infection IgM antibodies to 6 kD were not found. Changes in antibody production as measured by different ELISA techniques for IgG and IgM were not always reflected in changes of IgG or IgM blot band patterns.



INTERACTION OF PLASMODIUM FALCIPARUM OOKINETES WITH MOSQUITO MIDGUTS AND OOCYST DEVELOPMENT.

J.F.G.M. Meis, G. Pool, G.J. v.Gemert, A.H.W. Lensen and T. Ponnudurai

Department of Medical Parasitology, University of Nijmegen, The Netherlands.

It has been demonstrated in experimental P.bergei infections that ookinetes follow an intracellular route. However, it is not known whether such a route damages host cells. It has also been shown that there is increased mortality of mosquitoes infected with this parasite about the same time that ookinetes are passing through the midgut wall. Recent work on P.falciparum infections, however, has pointed to the possibility of intercellular migration of the ookinete. Ultrastructural studies demonstrated different stages of intercellular localization of the parasite. Further evidence of intercellular migration of P.falciparum ookinete has been produced using cytochemical staining with ruthenium red. Intercellular migration of ookinetes does not damage cell membranes and we do not observe increased mortality of P.falciparum infected mosquitoes during this time even with very heavy parasite loads. The difference in mortality rate of P.berghei and P.falciparum infected mosquitoes could therefore be related to the intra- or intercellular penetration of the ookinetes. More than 30 years ago, the avian parasite P.gallinaceum has been described as penetrating the midgut epithelium of Aedes aegypti intercellularly (1). It is therefore more than interesting, as it was shown recently that P.gallinaceum infected mosquitoes had no greater mortality than uninfected ones (2). The similarity between the mammalian parasite P.falciparum and the avian one P.gallinaceum is once again striking.

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PROTEIN COMPOSITION OF THE MALE AND FEMALE DIPETALONEMA VITEAE SURFACE

M.W.B.J. LAMERS, J.C.M. VETTER

Dept. of Medical Parasitology, University of Amsterdam, The Netherlands.

Evidence was obtained that Dipetalonema viteae antigens, circulating in the infected host, originate from the surface of the worm (CM Adema, et al; in press). Culturing male and female worms separately suggested that different products were released in the RPMI medium. These could be sex-specific surface antigens.

To gain knowledge about a possible difference in antigenic composition of male and female worms, the presence of these antigens in the surface was investigated. Also a possible antigenic or structural relationship between D.viteae and Onchocerca volvulus was sought for.

Because isolation of the cuticle was necessary and microdissection with this species was impossible, an extraction method was used (Betschart, et al). Chemicals were removed from the supernatant using a PD10 column and dialysis. The antigen solutions were electrophoretically analysed (SDS-PAGE). Mice were immunized with the thin male and female cuticle pieces. Sera were tested with IFAT on frozen sections of adult male and female D.viteae and O.volvulus. Further, these cuticles were solubilized using pancreatin; the resulting supernatant was also analysed in SDS-PAGE, after removal of the pancreatin by dialysis.

The mice sera give specific cuticle and epicuticle reactions in IFAT with D.viteae cross-sections. Sex cross-reactivity also occurs. Sera directed against female cuticle give stronger reactions, indicating a difference in composition of male and female cuticles.

In IFAT with O.volvulus cross-sections the sera show a specific hypodermal reaction, pointing at antigenic similarity between the D.viteae (epi)cuticle and the O.volvulus hypodermis.

SDS-PAGE of the extraction supernatant shows the presence of 51 female and 35 male bands, of which 32 female and 16 male are sex-specific proteins.

Dissolving the outer layers of the female cuticle with pancreatin gives 8 protein bands (25.5, 27.5, 32, 35.5, 37, 38, 41.5 and 46 kD) of which 3 (32, 35.5 and 41.5 kD) are sex-specific and 4 (27.5, 32, 35.5 and 46 kD) are cuticle-specific.

The dissolved male cuticle gives 6 protein bands (25.5, 27.5, 31.5, 37, 38 and 46) of which 1 (31.5) is sex-specific and 4 (27.5, 31.5, 37 and 46) are cuticle-specific.

Thus, the sex-specific cuticle proteins of the female worm are 32 and 35.5 kD and of the male worm 31.5 kD.

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MOLECULAR ANALYSIS OF BENZIMIDAZOLE RESISTANCE IN THE PARASITIC NEMATODE  
HAEMONCHUS CONTORTUS

M.H. ROOS, J.H. BOERSEMA, P.R. NILSSON, M. TAYLOR AND E.J. RUITENBERG.  
Division of Helminthology and Entomology, School of Veterinary Medicine,  
University of Utrecht, The Netherlands; Central Veterinary Institute,  
Weybridge, U.K.; National Institute of Public Health and Environmental  
Protection, Bilthoven, The Netherlands.

Anthelmintics, used against parasitic worms, interfere with parasite-specific processes. The study of the mechanism of anthelmintic resistance offers the opportunity to better characterize these parasite-specific traits and may open possibilities for a better drug design. As a model system the sheep intestinal parasite H. contortus is being used because this parasite easily becomes resistant to a number of anthelmintics. The resistance to benzimidazoles was chosen because resistance develops regularly, these drugs are widely used and new derivatives are being developed. Benzimidazoles are reported to interfere with several processes in the cell but evidence for interference with the microtubule assembly is the most convincing. Therefore we started with the characterization of the tubulin genes in normal and resistant populations. DNA from three normal and three resistant populations, isolated from different geographical locations, was isolated, digested with restriction enzymes, and analyzed by Southern blotting. As a probe a beta-tubulin gene, isolated from a genomic library we constructed from H. contortus DNA, was used. Normal populations were polymorphic for beta-tubulin, 2-6 bands were visible, depending on the restriction enzyme used. The different populations could be discriminated according to the banding pattern. Resistant populations showed 1-2 bands, identical in two populations and one population was different. The bands that were found in the resistant populations were already present in a low concentration in the normal strains. An in vitro selection experiment on the eggs of a normal population showed that after two generations the population had become resistant and that the tubulin DNA bands were reduced from 6 to 1. These results indicate that benzimidazole resistance is the result from selection of worms with a specific tubulin variant that are already present in the population.