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DR. H. J. VAN DER KAAJ in the chair

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COMMUNICATIONS OF THE POSTERSESSION

I. T. PONNUDURAI, A. D. E. M. LEEUWENBERG, and J. H. E. Th. MEUWISSEN
(Dept. of Parasitology, Faculty of Medicine, Catholic University, Nijmegen)

Plasmodium falciparum in culture

Several isolates of *P. falciparum*, from Africa imported into Holland, have been established in culture. One isolate has been in continuous culture for over 14 months.

Our principal objective with these cultures is the production of mature gametocytes infective to mosquitoes. The cultures were also used to study the chloroquine sensitivity of the different isolates and the susceptibility of erythrocytes from patients with hereditary spherocytosis.

Methods. A 9% suspension of human erythrocytes in RPMI 1640 medium supplemented with Hepes buffer, sodium bicarbonate and compatible human serum was held in plastic petri dishes as a thin settled layer. The cultures were maintained at 37° C in an atmosphere of lowered oxygen and raised CO₂ concentrations. This was initially achieved by the use of candle-jars and later in a modified CO₂ incubator supplied with premixed gases. Medium change was effected daily and Giemsa stained thin blood films made to monitor parasite growth. Fresh red cells were added every 4th or 5th day to give a starting parasitaemia of about 0.5%. Cultures were initiated from fresh isolates or cryopreserved stabiliates.

Chloroquine sensitivity tests were carried out by exposing the parasites to drug concentration ranging from 5 to 30 ng/ml over a period of 72 hours.

Results. Parasite growth was asynchronous and multiplication varied from 8–16 times over 96 hours depending on the isolate. Higher multiplication was obtained with lower starting parasitaemia, lower red cell concentrations or twice daily refreshing of medium.

Gametocytogenesis diminished over a period of time after the establishment of a culture from a fresh isolate. Factors required for gametocytogenesis were found to be different from those promoting gametocyte maturation. Although morphologically mature gametocytes were produced, infection of mosquitoes has not been achieved. In the assessment of chloroquine sensitivity it was discovered that the lethal dose, within the range tested, depended on the starting parasite density and the multiplication of the parasite.

Red cells from patients with hereditary spherocytosis supported parasite growth as well as normal cells.

2. S. MEIS, J. P. VERHAVE, P. JAP and F. HESS

(Departments of Medical Parasitology and Cyto-histology, Faculty of Medicine, Catholic University of Nijmegen)

Exoerythrocytic development of Plasmodium berghei in rat liver cells.

No information is available about the ultrastructure of the exoerythrocytic development, except for the mature schizonts inside parenchymal cells. We have initiated a study of the immature EEF before merozoite formation has started. Tedious as this may be, we attempted to facilitate searching, by restricting the liver volumes in which the sporozoites end up. 60% of the liver of young rats was temporarily ligated and the sporozoites were injected into the vena porta. Several biopsies were taken and finally the liver was perfused with glutaraldehyde at 43 hours.

The demonstrated results concern 43 h. EEF, not described this young before. The EEF are in a syncytial state and division spindles are visible in the nucleoids. The cytoplasm contains mitochondria of various shapes, with rare tubular or vesicular cristae. There are clusters of vesicular endoplasmic reticulum. No signs of further organelle formation were seen. The space between the hosts limiting membrane and the EEF-membrane is narrow. Unimembranous vesicles are seen all along the periphery of the parasite membrane, which contain fluffy material. They seem to fuse with, or pinched off, from the EEF-membrane, judging from their content, which is similar to that of the intermembranous space. There are also many invaginations seen into the host cell limiting membrane with the same fluffy content. It is still not clear whether this material is transported from the host to the parasite or *vice versa*. Finally it is striking that the cytoplasm of the host parenchymal cells lack the prominent α -glycogen, but otherwise it does not look exhausted.

The earlier stages of EEF-development and the host finding process of sporozoites are presently under study. Especially the latter mechanism is enigmatic, since sporozoites must somehow pass the endothelial lining of the sinusoids to contact their host cells. Considering the fact that bird-plasmodia grow inside endothelial cells, and that sporozoites of *Eimeria* are transported by macrophages to their development sites, we hypothesize that sporozoites of mammalian plasmodia pass through the liver cells or the Kupffer cells, on their way to the space of Disse and their final parenchymal host cells.

3. W. M. C. ELING

(Department Cytology and Histology, Faculty of Medicine, Catholic University of Nijmegen)

The role of T-cells during immunization against the rodent malaria parasite P. Berghei.

The majority of Swiss mice can be immunized against the virulent rodent malaria parasite *Plasmodium berghei* by the intraperitoneal inoculation of 10^7 parasitized erythrocytes (PE), followed 2 days later by a suppressive therapy (30 mg/l sulfadiazine in the drinking water) for a period 33 days and a challenge (10^5 PE) given 2 days after termination of therapy (Eling and Jerusalem, 1977). In the framework of the analysis of the various cell types involved in the induction of immunity against the parasite, sensitivity to treatment with antithymocyte serum was analysed. In these experiments ATS was injected during the course of the standard immunization procedure in separate phases, and each time in a separate group. The experiments showed a great sensitivity to an ATS dependent suppression of immunity to *P. berghei* close in time to the challenge infection. When ATS treatment was applied under a chemotherapeutic umbrella which prevented breakthrough of parasitemia and allowed recovery from the immunodepressed state, immunity was re-established. When ATS was administered 2 days or more after the challenge sensitivity to this agent has disappeared, and it became difficult to breakthrough immunity with ATS in the majority of challenged mice. These experiments point to a critical role for T-cells during the phase of induction of immunity. The challenge, completing the immunization procedure changes immunity either qualitatively i.e. it becomes independent of T-cells, or quantitatively i.e. T-cell numbers increase to such an extent that ATS cannot effectively interfere anymore.

Eling and Jerusalem *Z. Tropenmed. Parasitol.* 28 (1977) 158 and 293.

represent fertilized macrogametes; oocysts, however, were not observed. Our present observations confirm the absence of schizogony from the development cycle of this parasite in the final host.

Tadros W. and J. J. Laarman, *Proc. Kon. Ned. Akad. Wetensch. Ser. C*, 80 (1977) 351.

6. J. J. LAARMAN and WEDAD TADROS

(Dept. of Tropical Hygiene, Royal Tropical Institute, Amsterdam and Dept. of Parasitology, University of Amsterdam)

Light and electron microscopic studies on the cyst wall structure of several species of Sarcocystis.

The comparative morphology of the cyst walls of the following species of *Sarcocystis* was illustrated with the aid of light and electron micrographs:

species	intermediate host	definitive host	exhibited (light)	photographs (E.M.)
<i>S. suis</i>	<i>Sus scrofa domestica</i>	Primates	+	+
<i>S. miescheriana</i>	<i>Sus scrofa domestica</i>			
	<i>Sus scrofa ferus</i>	Canidae	+	+
<i>S. hominis</i>	<i>Bos taurus</i>	Primates	+	+
<i>S. cruzi</i>	<i>Bos taurus</i>	Felidae	+	—
<i>S. hirsuta</i>	<i>Bos taurus</i>	Canidae	+	+
<i>S. tenella</i>	<i>Ovis aries</i>	Canidae	+	—
<i>S. gigantea</i>	<i>Ovis aries</i>	Felidae	+	—
<i>S. cuniculi</i>	<i>Oryctolagus cuniculus</i>	Felidae	+	+
<i>S. putorii</i>	<i>Microtus arvalis</i>			
	<i>Microtus agrestis</i>	Mustelidae	+	+
<i>S. sebeki</i>	<i>Apodemus sylvaticus</i>	Strigidae	+	+
<i>S. cernae</i>	<i>M. arvalis</i>	Falconidae	+	+
<i>S. sp.</i>	<i>Mus musculus</i>	Strigidae	+	+
<i>S. sp.</i>	<i>Sorex araneus</i>	?	+	+
<i>S. sp.</i>	<i>Mustela nivalis</i>	?	+	—
<i>S. sp.</i>	<i>Cercopithecus aethiops</i>	?	+	—
<i>S. sp.</i>	<i>Cyanocorax chrysops</i>	?	+	+
<i>S. sp.</i>	<i>Alabuia multicarinata</i>	?	+	+

Our results indicate that most of the species examined can be readily distinguished apart on the basis of light microscopic examination (under oil immersion) of the cyst and cyst wall structure in fresh-mount preparation of micro-isolated sarcocysts; this method being superior to that of examining histological sections of infected muscular tissue. Fine structural features in common to all sarcocysts examined by electron microscopy in the present investigation include:

(i) The investment of sarcocysts with a primary cyst wall, consisting of a regularly interrupted osmiophilic layer, closely underlying a distinct unit membrane, which constitutes the boundary of the sarcocyst.

(ii) The extensive folding up of the primary cyst wall into numerous cyst wall protuberances, the arrangement and morphology of which appear to be largely species-characteristic and which obviously dramatically increase the total surface area of contact between the sarcocyst and the surrounding host tissue.

(iii) The presence of a layer of amorphous granular matter, which forms the core of the cyst wall projections, constitutes the bulk of the contents of very young cysts and which, in mature cysts, is

reduced to a thin layer along the periphery of the cyst and remnant strands amongst the cystozoites in the form of the septa.

It is interesting to note that, whereas elongate projections are common to the cyst walls of all 9 species studied, known to complete their sexual cycles in the intestinal tissue of mammalian carnivores, all three species which undergo gametogony in birds of prey (namely *S. sebeki*, *S. cernae*, *S. sp.* of *Mus musculus*) lack distinct elongate protrusions and exhibit instead remarkably similar, gently undulating cyst wall contours.

7. WEDAD TADROS, J. MARKS and J. J. LAARMAN

(Dept. of Parasitology, University of Amsterdam and Dept. of Tropical Hygiene, Royal Tropical Institute, Amsterdam)

Investigations on frenkeliosis and Frenkelia-induced coccidiosis in the Netherlands.

Enzootic areas of both the frenkelian species, that with lobulate cysts parasitizing *Microtus arvalis*, and that with the rounded cysts infective to *Clethrionomys glareolus*, were located in the Netherlands. The obligatorily heteroxenous eimeriid coccidian life cycles of both species have been confirmed in laboratory-bred voles and the common buzzard, *Buteo buteo*. The lobulate type of frenkelian cyst was successfully transmitted to the musk rat, *Ondatra zibethicus* by the oral administration of the corresponding sporocysts. Although *C. glareolus* was refractive to experimental infection with the lobulate type of cyst, we have to date recorded natural mixed infection with the rounded and lobulate types of cyst in 5 wild-caught bank voles.

Electron micrographs of brain cysts of both species, as well as schizonts lying in direct contact with the cytoplasm of parenchymal liver cells of *C. glareolus* were exhibited.

The successful vole to vole transmission of frenkeliosis by the intraperitoneal, but not by the oral, inoculation of liver homogenate, containing experimentally induced schizonts of the rounded type of frenkelian parasite is reported.

Repeated challenge of *B. buteo* with the frenkelian parasite of bank voles provided evidence for protective immunity in buzzards against reinfection with *Frenkelia*-induced coccidiosis, manifested as a steep decline in the duration of patency and a drastic reduction in the total output of sporocysts.

Studies on over 200 bank voles trapped, sexed, aged and individually investigated for the intensity of frenkelian infection, if any, and average diameter and, hence, estimated age of frenkelian brain cysts, when present, were carried out in an enzootic area, where *B. buteo* overwinters but is absent during spring and summer.

Results compiled over the last 2 years indicate:

- (i) no significant difference in incidence of frenkeliosis between male and female voles;
- (ii) the incidence of natural infection reaches 100% in voles trapped in February-March and is lowest in summer (June-August);
- (iii) more than 50% of naturally infected voles acquire frenkeliosis before attaining the age of 3 months;
- (iv) voles may acquire infection throughout the year, even in the absence of shedding buzzards from the area;
- (v) voles may become naturally reinfected, the number of cysts arising from the second infection being usually smaller than that of the primary infection.

8. WEDAD TADROS and J. J. LAARMAN

(Dept. of Parasitology, University of Amsterdam and Dept. of Tropical Hygiene, Royal Tropical Institute, Amsterdam)

Studies on the morphology, life cycles and biology of six species of Sarcocystis of small mammals in the Netherlands.

The life cycles of 6 species of *Sarcocystis* of small mammals have been investigated in our laboratory.

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as models for studies on diverse aspects of sarcosporidiosis and sarcocyst-induced coccidiosis of man and other large vertebrates.

S. cuniculi, parasitizing the musculature of the common European rabbit has been shown to undergo gametogony, unpreceded by schizogony, in the lamina propria of the small intestine of domestic cats 12 to 18 hrs p.i. Excretion of sporulated tetrazoic sporocysts ($12.6 \times 9.6 \mu\text{m}$) started 10 days p.f. and continued for up to 105 days. Maximum daily excretion per kitten reached 1.1×10^6 sporocysts. Challenge infection 4 months after the end of patency led to a steep decline in number of excreted sporocysts and a dramatic reduction in the period of patency. Many of the sporocysts excreted by challenged kittens were morphologically abnormal and apparently non viable. Experimentally infected laboratory rabbits developed similar I.F.A. titers against the homologous and a heterologous bovine cystozoite antigen.

S. putorii, parasitizing the common European vole, *Microtus arvalis* has been demonstrated to undergo gametogony in the lamina propria of the European weasel, the stoat and the ferret. Solid protective immunity, lasting for over a year, has been observed in weasels following challenge infection.

Comparative investigations carried out on the life cycles, light and electron microscopic morphology of 4 species of rodent *Sarcocystis*, with birds of prey as final hosts, namely: (a) *S. sebeki* of *Apodemus sylvaticus* which undergoes gametogony in *Strix aluco* (b) *S. cernae* of *M. arvalis* which completes its sexual development in *Falco tinnunculus* (c) *S. dispersa* of *Mus musculus* with *Tyto alba* as final host and (d) a new *Sarcocystis* species isolated from *Strix aluco* and which induces sarcocysts in *M. musculus*, revealed striking similarities. Thus: (i) the cysts of all 4 species attained a length of several mm, the development of the cyst being extremely rapid as compared to *Sarcocystis* species with mammalian final hosts. Young sarcocysts, containing metrocytes, were detected within muscle fibres as early as 12 days p.i. while sarcocysts with elongate cystozoites, infective to the respective final hosts developed 30 days p.i. (ii) all 4 species had conspicuously large metrocytes in young cysts, while elongate cystozoites were invariably under $10 \mu\text{m}$ in length and of a slender nature. (iii) the fine structure of the cyst walls of young sarcocysts of all 4 species was remarkably similar, exhibiting wavy undulations, but no conspicuous projections. (iv) large schizonts, reminiscent of those of *Frenkelia*, with a rosette arrangement of merozoites and absence of parasitophorous vacuole, were seen within parenchymal liver cells. Extensive damage to the liver, often with fatal outcome, ensued 5 to 7 days p.i. with heavy doses of sporocysts. (v) Exceedingly heavy parasitization of the intestinal lamina propria of tawny and barn owls as well as kestrels was repeatedly observed at autopsy of wild, naturally infected birds, the death of which could not be accounted for by bacteriological, toxicological or other obvious pathological causes.

9. M. H. MIRCK

(Dept. of Medicine, Veterinary Faculty, State University, Utrecht)

Some epidemiological aspects of a mixed strongyle infection in Shetlandponies in the Netherlands (Preliminary communication).

The whole year 1977 six natural infected Shetlandpony mares were allowed to graze a one hectare pasture. This pasture was divided into two equal parts; each part grazed upon alternatively during two weeks.

Fortnightly faecal and blood samples were taken from all six ponies. Also fortnightly pasture samples were collected separately from each part of the pasture. Mean strongyle egg counts rose slowly from January onwards and showed a steep increase in August. Egg output remained high till October when a steep fall was registered reaching an extreme low level in December.

Pasture larval counts showed a remarkable reduction of infective strongyle larvae in April, May and June. From July the larval counts increased to remain high during the rest of the year (maximum 141,000 strongyle larvae per kg dehydrated herbage). In April a sharp increase in mean leucocyte counts was found with over 10,000 leucocytes per mm^3 and remained high until October when the number of

leucocytes fell to normal values within one month. This pattern was caused by an increase of neutrophils and eosinophils between April and October.

The mean serum protein concentration increased in April and remained high (maximum 8.3 g%) until December. The mean β -globulin values decreased during August and September and rapidly reached a maximum in November (2.7 g%). In December the mean β -globulin concentration was normal again. In July, September and October the mean γ -globulin values were elevated (maximum 1.7 g%) and fell to normal in October and November.

10. E. A. MEULEMAN and R. C. PEET

(Laboratory of Medical Parasitology, Free University, Amsterdam).

The development of Schistosoma mansoni in Biomphalaria pfeifferi, with special reference to the ultrastructure of the daughter sporocyst body wall.

The essential changes marking the transition into parasitic sessile mother sporocyst occur within two hours after penetration of the miracidium (Meuleman *et al.*, 1978). The ciliated plates are shed into the haemolymph of the snail from which they are cleared by amoebocytes. At the same time the ridges between the plates expand to form the continuous outer layer which remains connected to sunken nucleated areas. The surface area of this tegumental structure becomes greatly increased to form a suitable interface with the host's interior. The germinal cells, which are embedded in parenchyma cells, start to divide. The parenchyma degenerates. When daughter sporocyst embryos develop from germinal cells they are enveloped by a so-called primitive epithelium which is formed by fusion of the extensions of the tegumental structure of the mother sporocyst. Somatic cells located peripherally in the developing daughter sporocyst expand and coalesce beneath the primitive epithelium to form the future outer layer of the tegumental structure of the daughter sporocyst. The primitive epithelium degenerates, the outer layer loses its nuclei and becomes connected to sunken nucleated cell bodies. Further developments in the tegumental structure of the daughter sporocyst include the formation of microvillus-like projections, a surface coat, spines and a basement membrane.

Following establishment of the daughter sporocysts in the digestive gland area their germinal cells give rise to the cercariae. The development of the cercarial body wall follows the same basic pattern as that of the daughter sporocyst except that parenchyma cells do not occur (Meuleman and Holzmänn, 1975).

Meuleman, E. A. and P. J. Holzman. *Z. Parasitenkd.* 45 (1975) 307-318.

Meuleman, E. A., D. M. Lyaruu, M. A. Khan, P. J. Holzmänn and T. Sminia. *Z. Parasitenkd.* 56, (1978) 227-242.

11. J. F. SLUITERS, R. C. PEET and E. W. ROUBOS

(Laboratory of Medical Parasitology, Faculty of Medicine, and Dept. of Biology, Free University, Amsterdam)

The effect of a Trichobilharzia ocellata-infection on the female gonadotrophic hormone producing Dorsal Bodies of Lymnaea stagnalis.

Infection of juvenile *L. stagnalis* with 4 miracidia of *T. ocellata* reduced growth of the ovotestis and of the female and male accessory sex organs, and prevented ovipository activity. However, gametogenesis and cellular differentiation of the accessory sex organs occur. It is suggested that the reproductive hormones are present, but that their secretion or the response of their targets is reduced by the parasite (Sluiters, 1978). In *L. stagnalis* vitellogenesis, cellular differentiation and growth of the female accessory sex organs and ovipository activity depend on the Dorsal Body Hormone (DBH) (Geraerts and Joosse, 1975). To study the effect of infection on Dorsal Body (DB) activity, a quantitative light and electron microscopical study was carried out using sections of DB's of snails dissected at day 22 post exposure. Compared to non-infected controls the DB cells of the infected snails showed a marked increase of the volume of cytoplasm, and of the number and volume of the Golgi zones. This indicates a hyperplasia of the DB cells and an increased rate of synthesis of DBH containing granules. Therefore it

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is concluded that *T. ocellata* stimulates DBH production in DB cells. In DB cell processes release phenomena of DBH were seen in both groups. With respect to the female reproductive activity these observations are not in agreement with the first part of the abovementioned hypothesis. However, it is not contradictory to the hypothesis that the reduced growth of the ovotestis and the accessory sex organs is exerted by the parasite by inhibiting the response of the targets to the reproductive hormones.

Geraerts, W. P. M. and J. Joosse *Gen. comp. Endocr.* 27 (1975) 450-464.

Sluiter, J. F. *Short commun. 4th int. Congress Parasit.*, (1978) Warsaw. Sect. F. p. 20.

12. A. G. M. TIELENS and S. G. VAN DEN BERGH

(Laboratory of Veterinary Biochemistry, State University, Utrecht).

In vitro excystation and energy metabolism of the juvenile liver fluke (*Fasciola hepatica*).

The adult common liver fluke (*Fasciola hepatica*) lives in the bile ducts of the liver where very little oxygen is available. It has a predominantly anaerobic energy metabolism in which glucose is fermented to form mainly propionate and acetate. The details of this fermentation process have been studied in this laboratory by Van Vugt *et al.* (1979). As the juvenile liver fluke lives in surroundings with higher oxygen levels, it might be expected to have an aerobic energy metabolism. The aim of this investigation is to test this hypothesis and, consequently, to study the biochemical regulation of the transition from the aerobic juvenile to the anaerobic adult stage.

In order to obtain large amount of juvenile flukes, a simplified method for the *in vitro* excystation of metacercariae (kindly supplied by Dr. H. J. Over of the D.C.I., Department of Parasitology, Lelystad). An excystation tower was designed with three compartments separated by small-mesh plastic netting. Bile and activated metacercariae are added to the top compartment. The newly excysted flukes can escape from the bile, which proved to be harmful for its further development, into the middle, bile-free compartment which is in contact with the air in the bottom compartment. The juveniles are harvested from the middle compartment, free from other materials.

It could be demonstrated that the juveniles derive their energy from endogenous glycogen stores. They utilize oxygen in a cyanide-sensitive fashion. Added glucose is aerobically broken down with CO₂ as the major end product. From these observations it is concluded that the juvenile fluke has a predominantly, if not entirely, aerobic energy metabolism.

Van Vugt, F., P. van der Meer and S. G. van den Bergh. *Int. J. Biochem.*, 10 (1979) 11-18.

13. Y. I. E. A. WETZLAR and J. N. VAN DER LINDEN

(Central Veterinary Institute, Dept. of Parasitology, Lelystad)

An attempt to demonstrate changes in intrahepatic portal venous circulation in infections with Fasciola hepatica in bovine.

On the basis of a comparative histological analysis of liver material from Frisian-Holstein calves artificially infected with *F. hepatica* and autopsied at 4, 8, 12, 16 and 20 weeks p.i. it seemed likely that part of the vascular changes might perform circularly disturbances.

In this respect it was remarkable that particularly the smaller calibres of the vena portae in the ventral lobe were affected. In the period 8-12 weeks p.i. vascular narrowing and thrombosis seem to demarcate the onset of collateral formation and anastomoses.

In an attempt to compare circulation in fluke infected and normal livers the portal system was perfused with Technovit[®] 1 under light pressure. After subsequent coagulation of the injected fluid the liver parenchyma was eroded with caustic potash. The remaining skeleton is a cast of the portal venous system and by this method the circulatory change in a limited number of animals was demonstrated.

Another way to show an eventually changed circulation is perfusing the portal system with barium sulphate. Perfusing pattern will be made visible by an X-ray of the liver.

The start of this last mentioned approach is made in cooperation with the Institute of Veterinary Radiology of the University of Utrecht.

¹ Technovit[®], Kulzer & Co. GmbH Bad Homburg, Western Germany

14. H. J. OVER, C. P. H. GAASENBEEK and N. NOORMAN
(Central Veterinary Institute, Dept. of Parasitology, Lelystad)

Environmental factors influencing the Oscillatoria-Lymnea truncatula-Fasciola hepatica-complex.

In the field the infection rate of *L. truncatula* populations and the infection level of individual snails with *F. hepatica* are low compared with the situation observed in the experimental model. As a rule in the field lower infection rates in the population are explained by reduced contact chances between miracidia of *F. hepatica* and *L. truncatula* and lower infection levels in individual snails are likely to be the result of unfavourable feeding situations in the field.

This last point however is not so clear as in experimental situations the relation food – snail growth – number of rediae – number of cercariae are equivocal. Snail density as such reversely influences the outcome of this process and moreover infected snails exhibit a reduced grazing activity.

The *Oscillatoria* spp.-*L. truncatula*-*F. hepatica*-complex might present an interesting model to analyze food chain efficiency in bio energyanalysis.

15. J. A. ZIJLMANS and M. H. MIRCK
(Dept. of Medicine, State University, Utrecht)

Spontaneous excretion of Cyathostominae in horses.

An investigation into spontaneous excretion of cyathostominae was performed in horses offered as patients at the Department of Veterinary Medicine from September 1975 till June 1978. A typical form of cyathostomiasis occurs in horses, mainly three years or less of age, during winter and early spring. Other striking clinical symptoms are debility, serious emaciation, bad appetite and frequently diarrhoea; this latter possibly caused by salmonellosis, which is often diagnosed at the same time.

Haematological examination of the patients reveals a rise of the β -globulin level from approximately 1.5 g% to about 2.5 g%, while the amount of albumin shows a decline from about 3.2 g% to 2%. Usually a raised leucocyte count is found, mostly due to neutrophilia. Faecal examination reveals a low or negative egg count and the presence of larvae, mainly fourth-stage, in maximum amounts of approximately 35 larvae per gram of faeces. Occasionally fifth-stage larvae and adult worms can be found.

Post-mortem examination shows a catarrhal inflammation of the large intestine, sometimes with haemorrhagic foci in the mucosa, and numerous coiled cyathostome larvae in the mucosa and submucosa.

16. F. H. M. BORGSTEEDE
(Central Veterinary Institute, Dept. of Parasitology, Lelystad)

Helminth parasites of wild foxes (Vulpes vulpes L.) in the Netherlands

During the period March 1978–September 1978, 71 foxes, mainly shot near the Dutch-German border, were examined for the presence of internal helminth parasites. The following parasites were found:

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Small intestine:

- Nematoda: *Uncinaria stenocephala* (54.9%)
Toxocara canis (67.6%), sometimes also in the stomach
Molineus patens (7.0%)
larval *Porrocaecum* spp. (1.4%)
- Trematoda: *Alaria alata* (8.5%)
Cryptocotyle lingua (4.2%)
Apophallus donicus (1.4%)
- Cestoda: *Taenia* spp. (57.7%)
Hymenolepis spp. (1.4%)

Lungs:

- Nematoda: *Capillaria aerophila* (35.6%)
Crenosoma vulpis (6.7%)

Urinebladder:

- Nematoda: *Capillaria plica* (5.3%)

No parasites were found in the liver and gallbladder.

An important result is the absence of *Echinococcus granulosus* in the examined material.

17. F. H. M. BORGSTEEDE and J. JANSEN

(Central Veterinary Institute, Dept. of Parasitology, Lelystad)

Spirurata in wild birds in the Netherlands

During a survey study of parasites in wild birds in the Netherlands the following Spirurata were found:

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|-------------------------------|-----------------------------------|
| <i>Gavia stellata:</i> | <i>Paracuari tridentata</i> |
| <i>Podiceps cristatus:</i> | <i>Decorataria decorata</i> |
| <i>Fulmarus glacialis:</i> | <i>Stegophorus stellaepolaris</i> |
| | <i>Seuratia shipleyi*</i> |
| <i>Phalacrocorax carbo:</i> | <i>Skrjabinocara squamata</i> |
| | <i>Desmidocercella incognita</i> |
| <i>Ardea cinerea:</i> | <i>Desmidocercella numidica</i> |
| <i>Botaurus stellaris:</i> | <i>Desportesius orientalis</i> |
| <i>Anas platyrhynchos:</i> | <i>Echinuria uncinata</i> |
| | <i>Tetrameres fissispina</i> |
| <i>Anas penelope:</i> | <i>Echinuria uncinata</i> |
| <i>Aythya fuligula:</i> | <i>Echinuria uncinata*</i> |
| | <i>Tetrameres fissispina*</i> |
| <i>Melanitta nigra:</i> | <i>Tetrameres fissispina*</i> |
| <i>Somateria mollissima:</i> | <i>Streptocara californica</i> |
| <i>Mergus serrator:</i> | <i>Streptocara crassicauda</i> |
| <i>Cygnus olor:</i> | <i>Echinuria uncinata</i> |
| <i>Buteo buteo:</i> | <i>Synhimantus hamatus</i> |
| | <i>Habonema leptoptera</i> |
| <i>Pernis apivorus:</i> | <i>Physaloptera apivora</i> |
| <i>Circus aeruginosus:</i> | <i>Synhimantus hamatus*</i> |
| <i>Falco peregrinus:</i> | <i>Synhimantus laticeps*</i> |
| <i>Falco tinnunculus:</i> | <i>Synhimantus laticeps</i> |
| <i>Haematopus ostralegus:</i> | <i>Streptocara crassicauda</i> |
| | <i>Cosmocephalus obvelatus*</i> |
| <i>Larus argentatus:</i> | <i>Paracuari tridentata</i> |
| | <i>Cosmocephalus obvelatus</i> |

<i>Rissa tridactyla</i> :	<i>Paracuarina tridentia</i>
<i>Uria aalge</i> :	<i>Streptocara crassicauda</i>
	<i>Paracuarina tridentata</i>
	<i>Cosmocephalus obvelatus</i>
<i>Asio otus</i> :	<i>Synhimantus laticeps</i>
<i>Asio flammeus</i> :	<i>Synhimantus laticeps</i>

An asterisk indicates a new host-parasite record.

18. H. A. VAN SEVENTER, C. C. M. KROON and E. N. SCHOEMAN; E. G. BOORSMA and H. J. VAN DER KAAJ
(Dept. of Parasitology, University of Amsterdam, Dept. of Tropical Hygiene, Royal Tropical Institute and Lab. of Parasitology, Medical Centre, University of Leyden)

Influence of aging of Anopheles atroparvus on susceptibility to infection with Plasmodium berghei berghei.

When selection was carried out for susceptibility or refractoriness to infection with *P. berghei* of *A. atroparvus*, only young mosquitoes of up to one week old were used. In fact only those mosquitoes that laid eggs after taking a single meal of *P. berghei*-infected blood were examined. A pathogen refractive (R) strain was selected. Several generations of subsequent selection of these refractory mosquitoes however did not result in a strain, fully resistant to infection with *P. berghei* (van der Kaay and Boorsma, 1977).

When three-weeks-old mosquitoes were infected, increased susceptibility was observed, irrespectively of whether mosquitoes were maintained on only raisins, glucose or were regularly given non-infected bloodmeals prior to infection. Thus for the R strain the percentage of refractory mosquitoes decreased from 94% (variation 100-84) when one-week-old mosquitoes were infected to 69% (var. 78-47) and 66% (var. 72-61) when mosquitoes were infected at the age of three weeks and fed prior to infection on raisins or blood. The mean number of oocysts increased to infection on raisins or blood. The mean number of oocysts increased from 6 to 17 and 18 respectively. In the non-selected laboratory strain (control strain) of *A. atroparvus* mosquitoes infected at an older age showed an increase in infection rate, but not in the mean number of oocysts.

The oldest mosquitoes obtained for infection, were field-caught specimens of *A. atroparvus*, collected at the end of their natural hibernation during the month of April. These mosquitoes, being at least seven months old, showed a considerable increase in infection rate up to 71% (var. 61-84), in comparison with their offspring infected at the age of one week.

The latter show an infection rate of 21% (var. 10-39). The mean number of oocysts increased from 40 (7-74) to 91 (var. 73-113). These findings indicate, that aging of *A. atroparvus* mosquitoes is accompanied by an increased susceptibility to an infection with *P. berghei*.

Van der Kaay, H. J. and L. Boorsma, *Acta Leidensia*, 45, (1977) 13-19.

19. J. J. MELLINK

(Laboratory of Medical Parasitology, Faculty of Medicine, Free University, Amsterdam)

The transmission of Venezuelan Equine Encephalomyelitis (VEE) to mice sensitized to mosquito bites by Aedes aegypti (L.)

The transmission of VEE to mice by *Ae. aegypti* was studied in relation to the hypersensitivity of the host to bites of the vector. Mice were sensitized by repeated exposures to bites. All mice thus treated exhibited type III - like cutaneous hypersensitivity reactions to challenge bites. Sera from these mice when tested against mosquito homogenates using the Ouchterlony-method, showed up to two precipi-

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iene, Royal Tropical Institute

infection with *Plasmodium*

infection with *P. berghei* of *A.*

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ity, Amsterdam)

) to mice sensitized to

the hypersensitivity of the

bites. All mice thus treated

bites. Sera from these mice

showed up to two precipi-

tation lines. In these sera no virus neutralizing antibodies could be found, such in contrast to other authors (Feinsod *et al.*, 1975a and b). Mosquitoes were infected by intrathoracic inoculation. After an incubation period this resulted in high levels of viral content of the saliva.

Three transmission-experiments were carried out. The conclusion drawn previously from the preliminary results of the first experiment could not be confirmed, that mice thus sensitized are partially protected against viral transmission by mosquito bites. The results of the second and third series suggested even the reverse, *i.e.* that in sensitized mice an infection becomes more easily established than in control animals.

It seems therefore that an inflammatory reaction at the site of inoculation of virus material might stimulate the infection process. On the other hand, it is still possible that under different conditions the presence of virus neutralizing antibodies induced by bites may offset the occurrence of such an event or may even lead to opposite effects.

My earlier findings (Mellink, *in press*) of a relationship between the duration of the penetration period of the infected mosquitoes and the transmission rates were corroborated.

Further research along these lines is in progress and its development was tendered for criticism at the meeting.

Feinsod, F. M., *et al.*, *Amer. J. trop. Med. Hyg.*, 24 (1975) 533-536

Feinsod, F. M., *et al.*, *Ann. N.Y. Acad. Sci.*, 266 (1975) 251-254

Mellink, J. J., *Trop. geogr. Med.* (in press)