ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 87

Schistosomiasis Japonica in the Pig

Aspects of Pathology and Pathogenesis

Maria Hurst

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

Schistosomiasis Japonica in the Pig

Aspects of Pathology and Pathogenesis

Maria Hurst

Akademisk avhandling, som med tillstånd av Veterinärmedicinska fakulteten vid SLU för avläggande av veterinärmedicine doktorsexamen, offentligen försvaras på engelska språket i Ettans föreläsningssal, Klinikcentrum, Ultuna, fredagen den 15 september 2000, kl 13.00.

Av fakultetsnämnden utsedd opponent: Professor Allen Cheever, National Institute of Health, Bethesda, USA.

Abstract

Schistosomiasis japonica, caused by the trematode *Schistosoma japonicum*, is a zoonotic disease with significant impact on public health in endemic regions in China, the Philippines and Indonesia. The pig is important in transmission of the infection, and is also used as an experimental animal in schistosomiasis research. The main objective of this thesis was to explore the pig as an animal model for pathological and pathogenetic aspects of human schistosomiasis japonica. Gross and histopathological changes in pigs after experimental infections of different intensity and duration were evaluated and related to parasitological variables. The hepatic egg granuloma was investigated with immunohistochemical methods. Naturally acquired infections in pigs were examined and compared to experimental infections. Lesions were essentially confined to the large intestine and liver. In the experimentally infected pigs, liver lesions were proportional in degree to the intensity of infection. Gross lesions in the intestine included multifocal areas of hyperaemia and haemorrhages, resembling those of acute human schistosomiasis japonica. Gross liver lesions were white nodules and fibrosis. Microscopically, several characteristic features of schistosomal hepatic fibrosis, including granulomatous obstruction of portal venules and periportal fibrosis, were present. Hepatic fibrosis was marked in early patency in the experimentally infected pigs with high intensity infections, and then regressed spontaneously. The degree of hepatic fibrosis was correlated with liver egg and granuloma density in both acute and chronic stages of infection, and liver egg density was correlated with faecal egg excretion in the acute stage. Faecal egg excretion could thus be used as an indicator of hepatic pathology in acute infections. The egg granuloma showed expression of MHC class II antigen and involved $CD4^+$ T cells, as well

as $CD8^+$ T cells, B cells and IgG. Signs of a modulated granuloma formation were apparent in the liver, but not in the intestine of the naturally infected pigs, and other organ-related differences in granuloma composition were also found. Self cure was observed in the experimentally infected pigs with high intensity infections, but not in the naturally infected pigs. Natural infection, in contrast to experimental infection, was associated with clinical disease and reduced weight gain in young pigs. The results indicate that the pig would be a useful animal model for studies of acute intestinal disease, early hepatic fibrogenesis, spontaneous resolution of hepatic fibrosis, and granuloma development and regulation. In addition, the pig could be used for examining the effect of a schistosome infection on the nutritional status of the host.

Keywords: *Schistosoma japonicum*, pig, pathology, granuloma, MHC class II, CD4, modulation, hepatic fibrosis, self cure, weight gain.

Author's address: Maria Hurst, Department of Pathology, SLU, Box 7028, S-750 07 Uppsala, Sweden.

Distribution:

Swedish University of Agricultural Sciences
Department of Pathology
Box 7028, S-750 07 UPPSALA, Sweden

Uppsala 2000
ISSN 1401-6257
ISBN 91-576-5944-3

Schistosomiasis Japonica in the Pig

Aspects of Pathology and Pathogenesis

Maria Hurst
Department of Pathology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2000

Acta Universitatis Agriculturae Sueciae
Veterinaria 87

ISSN 1401-6257
ISBN 91-576-5944-3

Tryck: SLU Service/Repro, Uppsala 2000

To My Boys

Abstract

Hurst, M. 2000. *Schistosomiasis japonica in the pig. Aspects of pathology and pathogenesis.*

Schistosomiasis japonica, caused by the trematode *Schistosoma japonicum*, is a zoonotic disease with significant impact on public health in endemic regions in China, the Philippines and Indonesia. The pig is important in transmission of the infection, and is also used as an experimental animal in schistosomiasis research. The main objective of this thesis was to explore the pig as an animal model for pathological and pathogenetic aspects of human schistosomiasis japonica. Gross and histopathological changes in pigs after experimental infections of different intensity and duration were evaluated and related to parasitological variables. The hepatic egg granuloma was investigated with immunohistochemical methods. Naturally acquired infections in pigs were examined and compared to experimental infections. Lesions were essentially confined to the large intestine and liver. In the experimentally infected pigs, liver lesions were proportional in degree to the intensity of infection. Gross lesions in the intestine included multifocal areas of hyperaemia and haemorrhages, resembling those of acute human schistosomiasis japonica. Gross liver lesions were white nodules and fibrosis. Microscopically, several characteristic features of schistosomal hepatic fibrosis, including granulomatous obstruction of portal venules and periportal fibrosis, were present. Hepatic fibrosis was marked in early patency in the experimentally infected pigs with high intensity infections, and then regressed spontaneously. The degree of hepatic fibrosis was correlated with liver egg and granuloma density in both acute and chronic stages of infection, and liver egg density was correlated with faecal egg excretion in the acute stage. Faecal egg excretion could thus be used as an indicator of hepatic pathology in acute infections. The egg granuloma showed expression of MHC class II antigen and involved $CD4^+$ T cells, as well as $CD8^+$ T cells, B cells and IgG. Signs of immunomodulation of the granuloma were apparent in the liver, but not in the intestine of the naturally infected pigs, and other organ-related differences in granuloma composition were also found. Self cure was observed in the experimentally infected pigs with high intensity infections, but not in the naturally infected pigs. Natural infection, in contrast to experimental infection, was associated with clinical disease and reduced weight gain in young pigs. The results indicate that the pig would be a useful animal model for studies of acute intestinal disease, early hepatic fibrogenesis, spontaneous resolution of hepatic fibrosis, and granuloma development and regulation. In addition, the pig could be used for examining the effect of schistosome infection on the nutritional status of the host.

Keywords: *Schistosoma japonicum*, pig, pathology, granuloma, MHC class II, CD4, modulation, hepatic fibrosis, self cure, weight gain.

Author's address: Maria Hurst, Department of Pathology, SLU, Box 7028, S-750 07 Uppsala, Sweden.

Contents

Introduction **11**
General background 11
Geographic distribution 14
History 14
Pathogenesis 15
Pathological manifestations 16
Disease syndromes and clinical signs 18
Immunopathology 20
Diagnosis 21
Treatment and control 22
Objectives of the study **25**
Materials and methods **25**
Animals and study designs 25
Parasites and experimental infections 26
Perfusion for worm recovery 27
Coprological examination 27
Tissue egg counts 28
Clinical pathology 28
Serology 28
Necropsy and histopathological laboratory procedures 28
Histopathological examination 29
Immunohistochemistry 29
Statistical methods 30
Results **31**
Experimental infections (Papers I-III) 31
Naturally acquired infection (Paper IV) 33
General discussion **36**
Method of experimental infection 36
Pathology of the intestine 36
Pathology of the liver 37
Morphology of the egg granuloma and organ-related differences 39
Immunoreactivity of the hepatic egg granuloma 40
Clinical disease and effect on growth 42
Self cure 43
Conclusions **44**
Suggestions for future research **45**

References **46**
Acknowledgements **58**

Appendix

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Willingham AL, Hurst M, Bøgh HO, Johansen MV, Lindberg R, Christensen NØ and Nansen P (1998): *Schistosoma japonicum* in the pig: The host-parasite relationship as influenced by the intensity and duration of experimental infection. *Am J Trop Med Hyg 58 (2)*: 248-256.

II. Hurst MH, Willingham AL and Lindberg R (2000): Tissue responses in experimental schistosomiasis japonica in the pig: a histopathologic study of different stages of single low- or high-dose infections. *Am J Trop Med Hyg 62 (1)*: 45-56.

III. Hurst MH, Willingham AL and Lindberg R: Schistosomiasis japonica in the pig: immunohistochemical characterisation of the hepatic egg granuloma (manuscript).

IV. Hurst MH, Shi YE and Lindberg R (2000): Pathology and course of natural *Schistosoma japonicum* infection in pigs: results of a field study in Hubei Province, China. *Ann Trop Med Parasitol 94 (5)*: 461-477.

Abbreviations

The following abbreviations are used in the text:

ELISA	enzyme-linked immunosorbent assay
EPG	eggs per gram
IFN	interferon
Ig	immunoglobulin
IHA	indirect haemagglutination
IL	interleukin
MHC	major histocompatibility complex
NK	natural killer
PI	post infection
SEA	soluble egg antigen
Th	T helper cell

Introduction

General background

Schistosomiasis, also known as bilharziasis, is one of the major parasitic diseases affecting man and animals in tropical and subtropical countries. It is caused by infection with trematodes belonging to the genus *Schistosoma* (blood flukes), which inhabit the vascular system of their final hosts. Schistosomes have an indirect life cycle with various species of fresh water snails as the intermediate host [143]. The habitats of the snails determine the geographic distribution of endemic schistosomiasis. The three major species that infect humans are *Schistosoma mansoni* and *S. japonicum*, which cause intestinal schistosomiasis, and *S. haematobium*, which leads to urinary schistosomiasis. *Schistosoma mansoni* and *S. haematobium* occur endemically in Africa and the Middle East, *S. mansoni* also in the Caribbean and South America, and *S. japonicum* in the Far East. In 1993, about 200 million people in 74 countries were estimated to be infected with schistosomes, and 500-600 million living in endemic regions were at risk of being infected [166]. The infection may lead to chronic ill health, and has serious consequences for socio-economic development in endemic regions [139].

Schistosoma japonicum is unique among the species that infect man in that it is zoonotic and also infects several species of domestic and wild mammals [70, 73, 98]. Domestic animals infected with *S. japonicum* contribute to contamination of the environment with schistosome eggs, and have an important role in maintaining transmission in endemic areas [57, 106, 127, 167, 177]. In addition, disease caused by *S. japonicum* in livestock leads to reduced productivity and considerable economic losses [43, 50, 51, 67, 146].

The major pathogenetic factors in schistosomiasis are not the worms, but their eggs, which trapped in the tissues of the host induce granuloma formation and fibrosis [156]. The most serious consequences of schistosomiasis japonica are seen in the liver, where a continuous influx of eggs with time leads to chronic fibro-obstructive disease and portal hypertension [138]. Schistosomiasis japonica has been studied experimentally in mice, rabbits and other laboratory animals and also in non-human primates [29, 156]. As models of human schistosomiasis, small laboratory animals have drawbacks, such as their small size and short life span relative to both humans and the parasites [11]. This makes it difficult to study the disease at infection levels comparable to those occurring in humans, and to carry out long-term studies [11, 23]. Among non-human primates, chimpanzees develop disease that is very similar to human schistosomiasis japonica, but their use in medical research is questionable for ethical and other reasons [93, 150].

The pig, which has many biological similarities with man, has attracted a lot of interest as a model of a variety of human diseases [150]. The need for an

alternative animal model in schistosomiasis research, together with the fact that the pig is a natural host for *S. japonicum*, provide the background for the exploration of the porcine model of schistosomiasis japonica that is the aim of the present thesis. The role of the pig in transmission of *S. japonicum* and the adverse health effects of the infection in pigs, are additional, important factors, which encourage studies of pigs in schistosomiasis japonica research.

Life cycle of S. japonicum *(Figure 1)*

Schistosoma japonicum is a digenetic trematode with a life cycle that comprises four stages: one stage within a definitive mammalian host for sexual reproduction, one stage within a snail intermediate host for asexual multiplication, and two free-living stages, the miracidium and the cercaria [143, 156]. The intermediate hosts for the different geographic strains of *S. japonicum* belong to the genus *Oncomelania,* which are small, amphibious fresh water snails able to survive for prolonged periods out of water. In addition to man, a wide range of domestic and wild mammals may serve as definitive hosts for *S. japonicum*, including cattle, water buffaloes, pigs, sheep, goats, dogs, cats and field rats [57, 70, 73, 77]. Laboratory animals, such as rodents and rabbits, are also susceptible to infection, as are several species of non-human primates.

The adult worms live in permanent pairs in the mesenteric veins of the definitive host, where they may survive for several years [62, 99]. The male is 10-20 mm long and about 0.55 mm wide, whereas the female is longer, 20-30 mm and only 0.3 mm wide. The female is able to produce 1000-3500 eggs per day [37], laid in clusters in small venules in the intestinal mucosa and submucosa [55, 113]. Each egg contains an embryo, which matures to a miracidium in 9-12 days and may survive in tissues for about 21-22 days [70, 113]. Histolytic enzymes secreted through the eggshell facilitate the passage of the egg through the vessel wall and the surrounding tissue to the intestinal lumen. The secretions induce an inflammatory reaction, promoting further tissue destruction. Eggs are expelled together with extravasated blood, tissue debris and inflammatory cells, a process believed to be facilitated by peristaltic bowel movements [55, 97]. The time interval between oviposition and egg excretion is about 6 days [11]. Once excreted from the host, eggs that reach fresh water will hatch to release the miracidium. Miracidial hatching is temperature-dependent (10 - 30°C), and is stimulated by a combination of light and changes in osmotic pressure [113, 143].

The free-living miracidium cannot feed and has to penetrate the correct snail host within a few hours after hatching [143]. Upon penetration of the snail, the miracidium changes into a primary sporocyst, in which secondary sporocysts form in 10-12 days. Numerous cercariae then develop within each secondary sporocyst and are shed from the snail. The prepatent period in the snail host varies with the outside temperature from 17-18 days at 30-35°C to several months at lower temperatures.

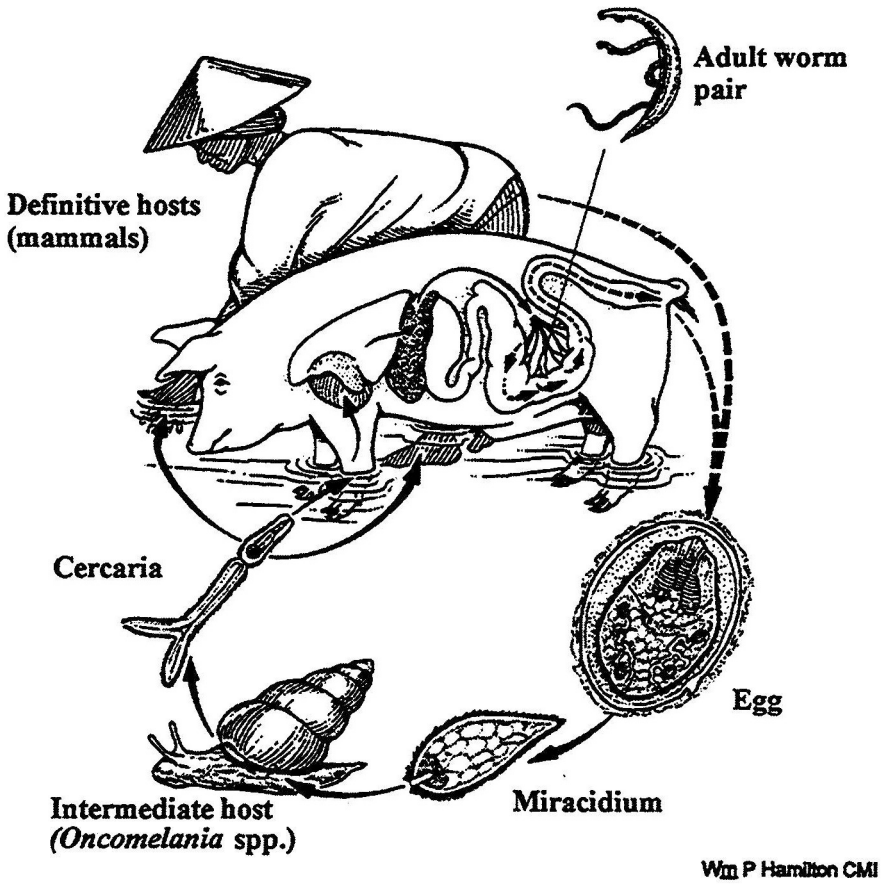

Figure 1. The life cycle of *Schistosoma japonicum*.

The cercaria is the infective stage for the definitive mammalian hosts. It swims vigorously in freshwater for only about 12 hours after shedding, but may survive for 2-3 days [97, 143]. On contact with skin, the cercaria secretes proteolytic enzymes to facilitate penetration through the skin. During penetration, it loses its tail and is transformed to the next larval stage, the schistosomulum, which migrates via the venous circulation to the lungs and then further to the systemic circulation. After a few days the schistosomulum reaches the portal system of the liver, where it matures to the adult stage in about 4 weeks. The male and female adult worms form permanent pairs for sexual reproduction, and move to their final habitat in the mesenteric and intestinal veins, where oviposition begins. The prepatent period for *S. japonicum* is on average about 42 days in humans [37]. In different experimental studies, the prepatent period was 42 and 36 days for buffaloes and cattle, respectively, 27-42 days for pigs, and 29-35 days for dogs [11, 68, 130, 165, 176].

Although the major route of infection with *S. japonicum* is through skin contact with infested water, infection via drinking-water may also occur [97, 114]. In addition, vertical transmission, leading to congenital infection of the foetus, is reported [109].

Geographic distribution

The main endemic regions for *S. japonicum* in China are the provinces Jiangsu, Anhui, Hubei, Jiangxi and Hunan in the marshland and lake district along the Yangtze river basin, and the mountainous provinces Sichuan and Yunnan [40, 135]. In the Philippines, endemic foci are found on Luzon, Mindoro, Samar, Leyte, Mindanao and Bohol islands [40, 87, 127]. In Indonesia, the infection is confined to Lindu and Napu Valleys in Central Sulawesi [20, 87, 88]. In Japan, *S. japonicum* used to be endemic on Honshu and Kyushu islands, but no new human cases have been recorded since 1978, and the disease is now considered to have been eradicated [145]. Two schistosome species, *S. mekongi* and *S. malayensis*, which resemble *S. japonicum*, occur endemically in Southeast Asia, but do not constitute a public health problem, since the number of infected people is limited and the morbidity is low [40].

History

Schistosomiasis japonica has occurred since ancient times in Asia [98]. *Schistosoma japonicum* eggs have been found in corpses buried over 2000 years ago in Hunan and Hubei provinces in China, and there are descriptions of clinical symptoms resembling acute schistosomiasis in Chinese medical literature from 400 BC [44, 98, 180]. In modern times, the first clinical description of schistosomiasis japonica was given by Fujii in 1847 in connection with an epidemic in the Katayama area, Hiroshima Prefecture in Japan, although he attributed the disease to the liver fluke *Clonorchis sinensis* [60]. Different Japanese scientists subsequently discovered schistosome eggs in human liver and in faeces [85, 96, 113] (**Figure 2**). The aetiology of the disease was finally established by the finding of adult schistosome worms in the portal veins of cats and humans [22, 83]. The percutaneous route of infection was demonstrated a few years later, and several additional species, notably cattle, horses, and dogs were found to be natural hosts [61, 97]. The life cycle of the parasite was established by the discovery of the snail host, *Oncomelania hupensis nosophora*, in 1914 [112].

In China, the disease was known under different names such as Yangtze Valley Fever and Hankow Fever after important endemic areas [55]. The first report of a human case of schistosomiasis japonica in China was published by Logan in 1905 [95]. Faust and Meleney [55] described the parasite and the disease in different definitive hosts, and discovered the intermediate snail host in China, *O. h. hupensis*. In the Philippines, human infection was first reported in 1906, and the

intermediate host, *O. h. quadrasi*, was discovered in 1932 by Tubangui [127]. In Indonesia, human cases of schistosomiasis japonica have been known to occur since the 1930's, but the intermediate host, *O. h. lindoensis* was not discovered until 1973 [19].

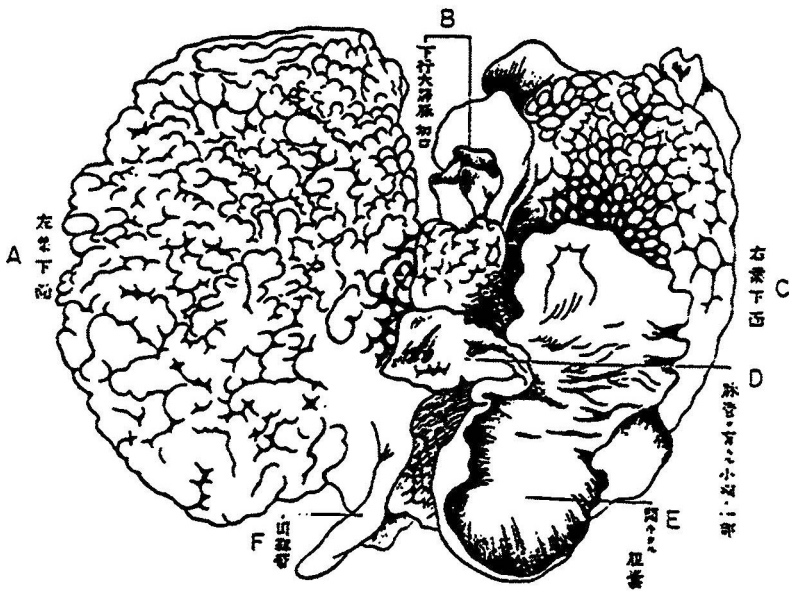

Figure 2. Human liver, chronic schistosomiasis japonica (Drawing from Majima 1888 [96]). This was the first recorded case of *Schistosoma japonicum* eggs in a human liver. (A) The left lobe, inferior portion; (B) The cut end of the portal vein; (C) The right lobe, inferior portion; (D) Vascular part of lesser omentum; (E) Opened gall bladder; (F) Hepatic ligament.

Pathogenesis

Pathological changes in schistosomiasis are caused by a variety of mechanisms, e.g. mechanical damage from cercarial skin penetration, larval migration, tissue reactions to killed organisms, and by antigenic or toxic secretions from the different developmental stages [156]. By far the most pathogenetic factor is the miracidium inside the egg. Eggs that fail to get excreted through the intestine remain in the gut wall, or are swept from the intestine via the venous blood to the portal system of the liver. Some eggs may reach the systemic circulation and are carried to the lungs, brain and other organs, or are laid directly at ectopic sites by aberrant worms [40].

The severity of the disease is influenced by the intensity and duration of infection, the parasite strain, and genetic factors and immune status of the host [37]. The most important disease manifestation in schistosomiasis japonica is seen in the

liver, where the egg-induced granulomatous inflammation and fibrosis of small portal radicles result in presinusoidal obstruction of the portal blood flow [144]. Eventually, periportal inflammation and fibrosis produce a characteristic lesion referred to as pipestem fibrosis, in which thick sleeves of fibrotic tissue surround the intrahepatic branches of the portal vein, largely with preservation of the structure of the hepatic lobules. The obstruction of portal blood flow leads to portal hypertension, splenomegaly, ascites and the development of a collateral circulation. The reduced portal blood flow is compensated by an increased arterialisation [4, 158]. Lesions induced by eggs in the lungs, brain and other organs may also be associated with clinical disease [40].

Pathological manifestations

Liver

In acute human cases, there may be miliary yellowish nodules on the external as well as on the cut surface [14, 67]. Liver enlargement, particularly of the left lobe, and increased firmness are typical of the early stage of chronic infection, but as the disease progresses the liver may become hard and decreased in size [67, 144]. The colour is dark brown due to accumulation of schistosomal pigment, and the surface in advanced cases is irregular with nodular swellings and deep scars. Pipestem fibrosis is generally seen on the cut surface [37, 144, 148]. There are few descriptions of the microscopic liver lesions of acute schistosomiasis japonica in humans. He and Zhu [67] describe intrahepatic congestion and diffuse inflammatory cell infiltration in prepatent infections. After the onset of oviposition, there is congestion, endophlebitis, inflammation of portal areas, eosinophilic egg abscesses and granulomas. Degeneration and ischemic necrosis of adjacent hepatocytes may occur. In human schistosomiasis mansoni, microscopic lesions, as observed in needle biopsy specimens from the liver of acute cases, were portal infiltration of eosinophils, histiocytes and lymphocytes, Kupffer cell hyperplasia, and only rare granulomas [3]. Focal single cell necrosis, hepatocellular cloudy swelling and vacuolisation were also seen. However, in a few autopsy cases of acute toxaemic schistosomiasis mansoni, there was massive dissemination of granulomas, often with a necrotic centre, in the liver.

In chronic cases, there is extensive fibrosis and increased vascularisation of portal areas, which may have an angiomatoid appearance, and numerous eggs are usually present [2, 144, 148]. Human livers have been found to contain between 2.1 and 881.5 million *S. japonicum* eggs [37]. Endophlebitis, focal endothelial proliferation, obstruction, and fibrous thickening of the wall of portal venules are common, and in some triads the portal venule may be absent [37, 102, 144]. The main portal vein is dilated and sclerotic, and may show thrombosis. Many eggs are calcified and induce little inflammatory reaction, but some are enclosed in egg granulomas, and embolised eggs in portal venules may also be found. Frequently interlobular septa are widened and fibrotic, with large numbers of calcified eggs [40, 67, 148]. The structure of the hepatic parenchyma is basically intact,

although there may be some degenerative hepatocellular changes and postnecrotic scarring, particularly near the surface of the liver [102].

Among experimental animals, only chimpanzees develop typical pipestem fibrosis, but portal pressure is not markedly elevated, due to the development of a collateral circulation [93]. Rabbits infected with *S. japonicum* develop marked periportal fibrosis resembling human pipestem fibrosis combined with cirrhotic changes after 2-3 months of infection [26, 149]. Despite dilation of porto-systemic collaterals, portal hypertension is not prominent in rabbits. *S. japonicum*-infected mice do not develop pipestem fibrosis, but get a marked elevation of portal pressure [155].

Intestine

In man, the worms tend to locate in the inferior mesenteric vein and the superior haemorrhoidal vein, resulting in a concentration of lesions in the large intestine, especially the rectum, sigmoid colon and descending colon [14, 39].

In the acute stage of infection there may be patchy areas of hyperaemia, haemorrhage, ulcerations and small yellowish nodules [63]. Small nodules with eggs may be found in the appendix. In experimental animals, lesions are in general multifocal or segmental with a macroscopically normal bowel in between lesions [29]. This pattern is explained by the tendency of *S. japonicum* worms to choose a few major egg-laying sites within the intestinal vasculature [28]. Lesions are characterised by a thickening of the intestinal wall and ulcerations [25, 55]. In rabbits, there may be sandy patches due to the presence of large numbers of calcified eggs, and large abdominal masses of eggs, inflammatory cells and fibrous tissue (bilharziomas) in mesenteric lymph nodes and the jejunal subserosa [27]. The location of lesions to a specific segment of the bowel seems to vary with the host animal species as well as the strain of *S. japonicum,* but small laboratory animals in general show more lesions in the small intestine, in contrast to large domestic animals and non-human primates in which lesions tend to be concentrated to the large intestine [29, 39]. Chronic intestinal lesions are characterised by mucosal hyperplasia, pseudopolyposis, ulcerations, thickening and induration of the intestinal wall, stenosis and, rarely, obstruction [35]. A correlation between chronic intestinal schistosomiasis and colorectal cancer exists, but the carcinogenetic mechanisms involved are not known [37, 78].

Microscopically, there are microabscesses containing egg clusters which may communicate with mucosal crypts, and perioval granulomas in the deeper layers of the intestinal wall [55, 156].

Spleen

In chronic schistosomiasis japonica, the spleen may be enlarged and congested, secondary to portal hypertension [37]. The capsule is often thickened and adherent to surrounding tissues. There may be reticuloendothelial hyperplasia,

follicular atrophy and fibrosis. Eggs and granulomas are rarely found in the spleen.

Lungs

Pulmonary haemorrhage caused by migrating schistosomula were noted in *S. japonicum*-infected rabbits and mice [55], and a marked inflammatory response to migrating schistosomula after repeated infections has also been observed in experimental animal studies [37]. Pulmonary obliterative arteriolitis induced by granuloma formation around embolised eggs occurs in man as well as in chimpanzees and other non-human primates [37, 93].

Brain

Initially, eggs may be detected in the leptomeninges and cerbral cortex [37]. Disseminated egg embolism leads to randomly distributed egg granulomas, but large solitary lesions characterised by granulomatous inflammation and large numbers of eggs are also found, presumably as a result of egg deposition by aberrant worms [131]. However, no adult worms have ever been detected in human brain. There is perivascular cuffing of plasma cells, eosinophils and lymphocytes [122]. Eggs are rarely found in the brains of experimental animals, although adult worms have been detected in monkeys and pigs [40].

Other organs

Immune complex-mediated glomerulonephritis, frequently seen in advanced cases of schistosomiasis mansoni, is apparently rare in schistosomiasis japonica [159]. It has been demonstrated in mice and rabbits, and renal amyloidosis may occur in the latter species [27, 94, 133]. Ectopic lesions due to dissemination of eggs via the systemic circulation may be found in almost all organs of the body, but are rare [40, 63].

Disease syndromes and clinical signs

Cercarial dermatitis

Cercarial penetration of the skin may result in local pruritus, erythema and papules. This is rather uncommon in endemic populations, but may occur in visitors from non-endemic regions [40, 63, 138]. Cercarial dermatitis is more often the result of penetration by cercariae of bird or rodents, a condition referred to as swimmer's itch [156].

Acute schistosomiasis japonica (Katayama fever)

Acute schistosomiasis japonica occurs after heavy infections, and the development of clinical signs usually coincides with the onset of egg production by the female worms [122, 138, 156]. It is clinically characterised by fever, chills, headache, general muscular pain, coughing, loss of appetite, nausea, abdominal pain and distension, and diarrhoea or dysentery with bloody, mucoid stools [37, 63, 122]. The liver may be enlarged and tender, and splenic enlargement may also

occur. Diffuse pulmonary infiltrates can be detected on radiological examination. There is always marked eosinophilia, and anaemia may develop as the disease progresses into the subacute or early chronic stage. Neurological signs indicating cerebral involvement are relatively rare, occurring in 2-4% of acute cases [63, 82]. Acute disease may occur as epidemics during the rainy season, especially in flooded areas [37].

Chronic schistosomiasis

Intestinal disease

There may be pain in the lower abdomen, and diarrhoea, with or without blood and mucus, is common [37]. Diarrhoea sometimes alternates with constipation, and an abdominal mass caused by thickening of the mesentery may be palpable.

Hepatosplenic disease

Hepatosplenic schistosomiasis may be associated with fatigue, weekness, abdominal pain and diarrhoea [135]. The liver, especially of the left lobe, is usually enlarged in the early chronic phase of hepatosplenic schistosomiasis [37]. Enlargement of the spleen may occur at this stage, but is more common in the later stage as a consequence of portal hypertension and passive congestion. Portal hypertension is associated with ascites, abdominal collateral vein dilatation and gastro-oesophageal varices, but liver function is usually not seriously impaired [144]. Haematemesis and melaena as a result of ruptured gastro-oesophageal varices are common, and bleeding episodes may be complicated by hepatic coma. Upper gastro-intestinal haemorrhage is a major cause of mortality in advanced, chronic human schistosomiasis japonica [37, 63, 144]. Severe hepatosplenic schistosomiasis was formerly common, but its prevalence is now reduced [135].

Other clinical manifestations

Cerebral involvement in schistosomiasis may be clinically manifested as meningoencephalitis in the acute phase of infection, and as epileptic seizures in the chronic phase [37, 131]. Some cases are asymptomatic. Lung involvement in the chronic stage of infection is rare, but if clinical signs are present, they are related to pulmonary hypertension and cor pulmonale [37, 63, 124].

Growth retardation

Schistosomiasis-related dwarfism, as a result of heavy or repeated infection in childhood, was formerly quite common in China [37]. These dwarfs showed signs of pituitary dwarfism, such as retarded physical growth and sexual development, in addition to other symptoms of schistosomiasis [67]. Dwarfism is now rare, but *S. japonicum* infection has been shown to be associated with retarded growth and development in children in China as well as in the Philippines [104, 105]. Growth reduction was manifested as decreased fat, muscle, and long bone growth, and was most marked during adolescence.

Immunopathology

Schistosomula and adult worms

Adult schistosomes evade the immune system of the host by incorporating host-derived macromolecules into their tegumental membrane and by losing expression of their own antigens [126, 141]. The larval schistosomula stage, in contrast, is vulnerable to attack by host immune mechanisms, and may be killed via antibody-dependent cellular cytotoxicity (ADCC) mediated by eosinophils and IgE, as well as via antibody-independent mechanisms [16].

The egg granuloma

Secretions from the miracidium within the egg induce a strong immune response, but the miracidium itself is protected by the egg shell. The egg shell functions as a nidus, around which macrophages and their derivatives, epithelioid cells and multinucleated giant cells accumulate and become organised [163]. Other inflammatory cells, mainly lymphocytes and eosinophils, but also neutrophils, mast cells and fibroblasts are involved in the egg granuloma. Fibroblasts produce a collagenous matrix that gives structural support to the inflammatory cells. The formation of a granuloma is a complex, dynamic process lasting several weeks, during which it undergoes stages of initiation, maturation and involution, and finally heals as the egg is destroyed, leaving a fibrous scar [74, 154, 163]. Granuloma formation is beneficial in that the granuloma protects the host from the harmful egg secretions and eventually destroys the egg, but detrimental in that it also leads to considerable tissue damage and fibrosis [91, 156].

Experimental studies in mice have shown that the *S. mansoni*-induced perioval granuloma is a manifestation of T cell-mediated delayed hypersensitivity [153]. It is dependent on MHC class II-restricted $CD4^+$ Th lymphocytes specific for egg antigens [71, 100]. Granuloma formation around mature viable eggs in murine schistosomiasis is vigorous in the acute stage of infection, but is reduced in the later, chronic stage, a phenomenon called immunomodulation [49]. Granuloma size is frequently used as an indicator of both hypersensitivity and modulation. Modulation is essential for the survival of the host, since the extensive tissue damage and fibrosis caused by the unmodulated, vigorous granulomatous inflammation leads to serious hepatosplenic disease [33, 155].

$CD4^+$ Th cells can be divided into two subsets, Th1 and Th2, depending on the cytokine profile that they are associated with. Th1 responses are accompanied by secretion of IL-2 and IFN-γ, and Th2 responses are characterised by secretion of IL-4 (promoting IgE production), IL-5 (stimulating eosinophil differentiation), and IL-10 [34]. Vigorous granuloma formation in murine schistosomiasis mansoni is associated with a Th2-like cytokine profile, whereas a Th1-like cytokine profile is associated with formation of smaller granulomas. The down-regulation of granuloma size is complex. Several mechanisms may be involved,

such as $CD8^+$ T suppressor cells and NK cells, at least partly via secretion of IFN-γ, B cells through an Fc- receptor dependent mechanism, antiidiotypic T cells, and immune complexes via downregulation of MHC class II [1, 66, 79, 128, 132].

The murine *S. japonicum* egg granuloma is also T cell dependent and involves a Th2-like cytokine profile in the acute stage of infection [142, 171, 174]. Modulation of the murine *S. japonicum* egg granuloma in chronic infections is believed to be mediated mainly via regulatory cross-reactive antibodies, whereas cellular mechanisms have a regulatory role in the acute phase of the infection [30, 120, 121].

Fibrosis

Most pathology associated with chronic schistosomiasis is due to fibrosis, which in mice is directly associated with the egg granulomas [8, 33]. Fibrosis is a dynamic process of deposition and resorption of extracellular matrix constituents, e.g. glycosaminoglucans and collagens [110]. Collagen synthesis and degradation peak when the granulomas are most vigorous and decrease in the chronic phase. The immunoregulation of fibrosis is probably partly independent from that of the granulomatous inflammation [32, 86]. Imature fibrous tissue is rapidly degradable, when the initiating cause is removed, whereas mature collagen is stabilised by cross-linking molecules, which block collagenolytic enzyme activity, rendering the tissue more resistant to degradation [8]. Murine *S. japonicum*-induced hepatic fibrosis has been shown to regress progressively after treatment with praziquantel [6], and hepatic pathology in humans may also be slowly reversible after chemotherapy [18]. However, advanced pipestem fibrosis may not be improved by treatment [119].

Diagnosis

Direct parasitological methods

Several methods can be used for the diagnosis of schistosomiasis japonica. Direct parasitological methods detect eggs in faeces or tissues and are highly specific [37]. A commonly used method for stool examination is the modified Kato-Katz thick-smear technique, which is quantitative and suitable for population-based surveys of prevalence and intensity of infection [84, 135]. The sensitivity of methods based on faecal egg detection may be low and light infections may not be detected. The miracidial hatching test is a non-quantitative, but sensitive test for detection of viable eggs [135]. Microscopic examination of rectal biopsies for detection of eggs is a highly specific and sensitive method, but the invasive technique limits its large-scale use [37]. Eggs or parasites may also be detected in biopsies from other organs, especially the liver [88].

Indirect methods

Indirect diagnostic methods detect pathological changes induced by infection. Hepatosplenomegaly can be diagnosed by clinical examination [37]. Liver biopsy

may reveal characteristic granulomas and fibrosis [2, 88, 144]. Various biochemical markers, e.g. procollagen III, hyaluronic acid, collagen IV and laminin may be used to assess liver fibrosis [135]. Imaging techniques, such as ultrasound, magnetic resonance imaging, and computer tomography scanning are used to detect typical hepatic lesions [17, 118, 160], and also to monitor their post-treatment resolution [119].

Serological methods

Serodiagnostic methods measure the immune response to egg and worm antigens. A range of antigens and test systems may be used, such as the circumoval-precipitin test, ELISA, IHA, and indirect immunofluorescent assay [37]. These tests are as a rule of higher sensitivity, but lower specificity than stool examination tests, and past and present infections cannot be differentiated. In contrast, circulating schistosome antigens can be used as markers for an ongoing infection, as well as for evaluation of the effect of chemotherapy and different assays have been developed, but are not yet in large-scale use [135].

Treatment and control

Chemotherapy

Various drugs, e.g. trivalent antimonials and furapromidum, have been used for treatment of schistosomiasis japonica in the past, but are now largely discarded due to long treatment periods, impractical administration procedures, and toxicity [36]. Praziquantel, developed in the late seventies, is now the preferred drug due to its efficacy, easy administration, and low toxicity [45, 139]. It is effective against the early schistosomula stage and adult worms and also kills mature eggs present in the tissues of the host by inducing miracidial hatching, but has little effect on the developing larval stages 3-21 days after infection [101, 135]. The current community-based treatment regime is a single oral dose of 40 mg/kg for humans and 25 mg/kg for bovines [90]. Pigs have been successfully cured of experimental *S. japonicum* infection with a single oral dose of 40 mg/kg [81]. Treatment leads to parasitological cure and reduced morbidity, but offers no protection against reinfection [90].

Chemoprophylaxis

Since praziquantel has no effect on maturing schistosomula, it cannot be used prophylactically. An antimalarial drug, artemether, has been shown to be effective against the larval schistosomula stage in animal experiments, and its usefulness as a chemoprophylactic agent in humans has been assessed in field trials [170]. It was found that artemether could prevent the establishment of a patent infection in high-risk groups, such as flood-control workers, and thus may become an additional tool in schistosomiasis control. However, the risk of drug resistance developing in malaria parasites must be considered before artemether is used for chemoprophylaxis in areas endemic of both schistosomiasis and malaria [170].

Control

Control of schistosomiasis is aimed at reducing the transmission as well as the morbidity of the infection [161, 164]. Transmission can be interrupted by reducing water contact of definitive hosts and by reducing contamination of the water with eggs. Provision of safe water, improved sanitation, health education and community participation are all important factors for achievement of this aim [138, 139, 161]. Snail control by the use of molluscicides and by modification of the environment to decrease snail habitats also diminishes transmission [90]. Chemotherapy is not only instrumental in reducing morbidity, but also contributes to reduced transmission levels by stopping or lowering egg output in treated individuals [47].

In the past, schistosomiasis japonica was a disastrous public health problem in China with over 10 million infected, and this led to the establishment of a national control program in 1955 [41]. Control mainly based on snail control, chemotherapy and health education has since then led to eradication of schistosomiasis in some parts of China, and greatly reduced levels of infection in others [134, 139]. About 0.9 million people are currently infected with *S. japonicum* in China [42]. However, control of schistosomiasis japonica is extremely difficult due to the wide range of animal species that are naturally infected. Domestic animals, especially cattle, buffaloes and to some extent pigs, are the most important reservoir hosts for humans in China, and over 100 000 bovines are at present estimated to be infected [90]. Current control programs in China take this into account and include chemotherapy with praziquantel for both humans and livestock [80, 90, 167, 177].

A substantial threat to schistosomiasis control in China is posed by the construction of the Three Gorges Dam on the Yangtze river [140]. This dam will create a 600 km long lake upriver that will be located between the two major transmission zones for *S. japonicum*. It is feared that the parasite and the intermediate host might be introduced into the lake as a result of migration of people and increased river traffic, and once established it would be very difficult to eradicate [135]. Transmission patterns may change in the down-stream *S. japonicum*-endemic region as well due to the marked ecological changes caused by the dam.

In the Philippines, the prevalence and intensity of infection have decreased, mainly as a result of large-scale chemotherapy, but over 0.5 million people are still estimated to be infected, and 10 million live in endemic areas [123]. Field rats are considered to be the most important reservoir hosts in the Philippines [57].

Vaccine development

Chemotherapy with praziquantel is highly effective, but needs to be repeated frequently due to reinfection [90]. This is costly and increases the risk of *S.*

japonicum resistance to praziquantel. Considerable efforts are therefore put into the development of a vaccine against schistosomiasis japonica for livestock as well as humans [108, 146]. Various levels of immunity have been induced in cattle, buffaloes and pigs by vaccination with attenuated cercariae or schistosomula [76, 136, 137, 172], but these vaccines are impractical for large-scale use. Promising results have been achieved using defined native and recombinant *S. japonicum* antigens, e.g. paramyosin and glutathione-S-transferase, especially in buffaloes, sheep and pigs (for a review, see [108]). No vaccine candidate has yet given complete protection, but even partial protection induced in livestock, especially bovines, is expected to have a major impact on human transmission.

Objectives of the study

The overall objective of the present work was to explore the pig as an animal model for pathological and pathogenetic aspects of human schistosomiasis japonica. More specifically, the objectives were:

- To study gross pathological and histopathological changes after experimental *S. japonicum* infections of different intensity and duration, and relate these changes to parasitological variables.

- To characterise the immunomorphology of the *S. japonicum* egg granuloma in the pig in acute and chronic stages of infection.

- To investigate the course and pathology of naturally acquired schistosomiasis japonica in pigs and compare this with experimental observations.

Materials and methods

This thesis is based on three studies of experimentally *S. japonicum* -infected pigs from two experiments, experiment A (papers I-II) and B (paper III), and one field study of naturally infected pigs (paper IV). A summary of the materials and methods used for each study is presented here. For detailed descriptions, see the different papers.

Animals and study designs

Papers I-III

The pigs used for experimental infections were Danish Landrace/ Yorkshire/ Duroc cross-bred pigs, raised and kept at the Specific Pathogen-Free (MS-SPF) swine research farm Sjaelland III, Denmark. The MS-SPF designation implies that the pigs are free from infection with *Actinobacillus pleuropneumoniae*, swine dysentery, atrophic rhinitis, mange and lice, but not from infection with *Mycoplasma hyopneumoniae*. The pigs were housed in standard pens under helminth-free conditions, although all harboured *Balantidium coli* infections, and were fed a standard ration of ground barley with water provided *ad libitum*. Cercarial exposure procedures were carried out at the swine research facility. At the end of the experiments, the pigs were moved to the Royal Veterinary and Agricultural University for perfusion and/or necropsy. The pigs used in these experiments were treated in accordance with animal ethics laws of Denmark.

Experiment A was designed to examine the effects of infection intensity and duration on different parasitological, clinico-pathological and pathological variables (papers I and II). To achieve this, a total of 96 pigs aged 6-10 weeks

were infected with 0, 100, 500 or 2000 *S. japonicum* cercariae, and subgroups were perfused and necropsied 4, 11, 17 or 24 weeks PI. Faecal samples for parasite egg counts were collected from each pig every 2 weeks. The pigs that were perfused at 24 weeks PI were weighed and had their blood sampled every two weeks throughout the experiment. Only the 0, 100 and 2000 cercariae dose groups were included in the histopathological study (paper II).

The purpose of experiment B was to analyse the immunomorphological dynamics of egg granulomas at different timepoints after a single infection and to examine the possible effects on the immunomorphology of a challenge infection. A total of eight pigs, aged 8-12 weeks at the beginning of the experiment were each infected with one or two doses of 850 S. *japonicum* cercariae. Four pigs were infected in week 0 and were necropsied at 12 or 21 weeks PI. Two were infected in week 0, challenged in week 12 and necropsied in week 21, and two were infected in week 12 and necropsied at 21 weeks PI. Four additional pigs of the same age served as uninfected controls and were necropsied at 12 and 21 weeks respectively.

Paper IV

The field study was set up to follow the development of infection in pigs naturally exposed to *S. japonicum* daily for one transmission period (April – October) in a highly endemic region in China, and to examine the resultant pathology at the end of the season. A farm in Hubei province in the Yangtze River basin was chosen for the study, in which a total of 19 Landrace pigs born and raised on this farm were used. The exposed pigs were divided into two groups, group A with 5 pigs aged 5 months and weighing about 50 kg, and group B with 10 piglets aged 8 weeks and weighing about 21 kg. Group A had previously been exposed to *S. japonicum*, and were seropositive, although faecal egg negative, at the start of the study, whereas group B were schistosome naive. The four remaining pigs were unexposed controls. The pigs that were exposed to *S. japonicum* were allowed onto the *Oncomelania* snail-infested pasture during daytime and housed in pens at night, whereas the unexposed controls were kept penned throughout the study. The pigs were fed a commercial, pelleted complete pig feed, and regularly observed. Clinical signs were recorded and rectal temperatures measured. Blood samples for serology were collected on day 1, 34, 82 and 126, and faecal samples at the same timepoints and on day 154. At the completion of the study, the pigs were weighed and transported to a research farm for slaughter and necropsy on days 167-169.

Parasites and experimental infections

Papers I-III

Two different Chinese mainland *S. japonicum* isolates were used for the experimental infections. These isolates, originating from Anhui and Zhejiang provinces in China, respectively, were maintained in *Oncomelania hupensis hupensis* snails and passaged in mice at the Danish Bilharziasis Laboratory. Snails

were exposed to miracidia hatched from eggs recovered from the livers of infected mice, and cercariae were obtained by shedding pools of the infected snails. The pigs were infected via intramuscular injection of cercariae suspended in Iscove's medium.

Perfusion for worm recovery

Papers I and II

The pigs in experiment A were perfused at necropsy for the recovery of worms from the liver and intestines. In order to achieve a hepatic shift of the worms, the pigs were given praziquantel (40 mg/kg) orally one hour before perfusion, and were sedated 30 minutes later with azaperonum (4 mg/kg) i.m. The pigs were killed by an overdose of pentobarbital (30 mg/kg) i.v. after administration of heparin (500 IU/kg) i.v. The perfusion fluid was a sodium-citrate buffer with sodium nitroprusside added as a vasodilator. Perfusion fluid was collected via a tube inserted into the portal vein and passed through a 45 μm sieve for collection of the worms. The liver and intestine were perfused separately via the hepatic vein and cranial mesenteric artery, respectively. The perfused worms were counted according to sex and maturity. The entire intestinal tract was then examined for residual worms, and their number, sex, viability and location were recorded. The percentage worm recovery in relation to the initial cercarial dose was calculated.

Coprological examination

Papers I and II

Faecal egg counts were determined by a combined filtration and sedimentation/-centrifugation method [56, 151]. A 5 g sample was washed with 1.2% saline through a series of 3 sieves with decreasing mesh sizes of 400, 100 and 50 μm. The material thus collected was washed and sedimented twice, and the remaining sediment was centrifuged. One fifth of the sediment was removed by a pipette and examined for schistosome eggs, giving an egg count equivalent to EPG faeces. The McMaster technique was used to examine the faeces of all pigs for other gastrointestinal helminths at the beginning and end of the experiment.

Paper IV

A miracidial hatching test was used to detect excretion of viable eggs. This test, which is not quantitative, is routinely used when <50 eggs per gram are found in faecal samples at the Tongji Medical University, China, where the laboratory part of the study was carried out. Fifty g faeces were homogenised in saline, strained through gauze, and allowed to sediment. After replacement of the saline with distilled water, the suspension was kept in a flask for several hours at room temperature. The neck of the flask was then examined with a magnifying glass to detect free-swimming miracidia. Salt flotation was used to detect other parasite eggs and protozoa.

Tissue egg counts

Papers I, II and IV

The number of eggs per g (EPG) liver was determined by digestion of a 5 g-sample of liver tissue in 3% KOH at 37° for 18 hr (papers I-II) and in 5% KOH at 37°C for 2 h or at 4°C over night (paper IV), after which the released eggs were counted using a counting chamber.

Clinical pathology

Paper I

Standard laboratory procedures were used to assess haematological variables, i.e. packed cell volume, haemoglobin concentration, erythrocyte, total leukocyte and eosinophil counts, and serum albumin concentration, in experiment A.

Serology

Paper IV

Serum samples from each pig were examined with an IHA test for detection of *S. japonicum* SEA-specific antibodies [54] and with an ELISA for detection of antibodies to both SEA and *S. japonicum* adult worm antigen [21].

Necropsy and histopathological laboratory procedures

Papers I, II and IV

After the animals were killed, and after perfusion (papers I and II), the organs were removed and examined. Gross lesions were recorded and graded as mild, moderate or marked. Tissue samples were collected from several organs and fixed in 10% neutral buffered formalin. Intestinal samples were collected from predetermined segments, and within these, preferentially from areas with gross lesions and in areas corresponding to mesenteric veins with adult worms or their remnants. The samples were trimmed, conventionally processed and embedded in paraffin. Sections, 4 μm thick, were cut and routinely stained with haematoxylin and eosin, and examined by light microscopy. Additional stains used were Masson's trichrome for collagen, resorcin-fuchsin for elastin and Gomori's stain for reticulin.

Paper III

Tissue samples from the liver, portal lymph node and spleen were fixed in 10% neutral buffered formalin and processed and embedded as above, and serial 4 μm sections were cut. Small pieces of the same organs were mounted on strips of filter paper, snap frozen in liquid nitrogen, and stored at –70°C. Prior to cryostat sectioning, the samples were embedded in O.C.T. compound (Miles Laboratories Inc., Elkhart, IND, USA), after which 4 μm thick serial sections were cut. The first and last section of each series of liver tissue sections, comprising approximately 10 cryostat or paraffin sections, were stained with haematoxylin

and eosin for morphological assessment, and the sections in between were used for immunostaining.

Histopathological examination

Paper II

Eggs present in liver and intestinal sections were counted, classified as intact or not intact, and the tissue response to eggs was characterised as either an acute inflammatory focus or as a granuloma. Eggs without or with only minimal tissue responses were recorded as free eggs. The proportions of free eggs versus eggs in tissue reactions were calculated. The composition of acute inflammatory foci and granulomas was recorded in detail. Granuloma diameters were measured with an ocular micrometer. The distribution of eggs and lesions in the intestinal wall was recorded. For the liver, the degree of periportal and septal fibrosis was scored as 0=none, 1=mild, 2=moderate and 3=marked, and the sum of the two scores was used as a measurement of liver fibrosis. Granuloma density was estimated by calculating the number of granulomas /cm^2 of liver tissue section using a semiautomatic digital image and analysis system (Imaging System KS 100, Kontron Elektronik GmBH, Eching, Germany).

Paper III

Based on the results of paper II, liver granulomas were classified according to developmental stage into exudative-productive, productive or involutional stages (see Results). Only centrally sectioned granulomas with eggs were examined. The number and condition of the eggs and the proportion of all cells in each granuloma that were eosinophils were noted, and significant fibrosis was recorded. At least 25 granulomas per pig were examined in the cryostat and paraffin sections, respectively.

Paper IV

The number of egg clusters, either free of tissue reaction or enclosed in acute inflammatory foci, and the number of granulomas in the intestine were recorded for each pig. Granulomas in the intestine and liver were classified according to developmental stage as in paper III, and the predominant type of inflammatory cell as well as significant fibrosis were recorded. Granuloma density in the liver was estimated from the number of granulomas per microscopic low-power field.

Immunohistochemistry

Paper III

Cryostat sections were fixed in acetone and incubated with 20% normal goat serum to reduce non-specific protein binding. The sections were then incubated with monoclonal antibodies against porcine MHC class II, wCD21, γδ T cell receptor, CD4a, wCD8b and wCD25, and with a polyclonal anti-human CD3ε antiserum, using the alkaline phosphatase-conjugated EnVision™ method (DAKO A/S, Glostrup, Denmark). The sections were developed with Vector®Red

Alkaline Phosphatase Substrate Kit (Vector Laboratories, Burlingame, CA, USA). Paraffin sections were pre-treated with 0.05% pronase for antigen retrieval and with 3% H_2O_2 to quench endogenous peroxidase, and immunostained with polyclonal antisera against porcine IgG, IgA and IgM by the streptavidin-biotin-complex/horseradish immunoperoxidase method. The sections were developed with 3,3' diaminobenzidine tetrahydrochloride (DAKO). All sections were counterstained with haematoxylin, permanently mounted with xylene-soluble medium, and examined by light microscopy. The immunoreactivity of all primary antibodies was assessed in either lymph node or spleen tissue, and optimal dilutions were determined. Replacement of the primary antibodies with an equally diluted negative control serum was used as a regular specificity control protocol. In each pig, the first 25 of the histologically classified granulomas that were encountered in the immunostained sections from the same series were examined. The proportion of the granuloma cells that were immunostained, and the principal location of the stained cells were determined. The average proportions of $CD4^+$ and $CD8^+$ cells for each granuloma stage and timepoint were compared.

Statistical methods

For all statistical tests used, P-values < 0.05 were considered significant.

Paper I

One-way analysis of variance was used to test for differences between subgroup means of parasitological and clinicopathological parameters. Pairwise comparisons of the means were done using Scheffe's range test. The relationships between numbers of worm pairs, faecal egg counts and liver egg counts were investigated with Pearson's correlation test.

Paper II

Two-way analysis of variance was used to test for differences between subgroup means of histopathological and parasitological parameters. Figures for liver EPG and granuloma density were adjusted for the growth of the pigs, assuming liver weights to be 1.7% of the total body weight at any timepoint [59]. The relationships between scores for liver fibrosis, and granuloma density and liver EPG, respectively, were investigated with Spearman's rank correlation test.

Paper IV

The weight gain of the exposed pigs were compared with that of the controls using one-way analysis of variance.

Results

Experimental infections (Papers I-III)

Parasitology and clinical pathology (Papers I and II)

All *S. japonicum*-infected pigs developed patent infections, and the prepatent period was 6 weeks in most of the pigs. Clinical signs were limited to diarrhoea in connection with initial egg excretion, and weight gain was not affected by the infection. Faecal egg excretion peaked at 8-10 weeks PI and was significantly higher in the 2000 cercariae dose group than in the two lower dose groups at 6-14 weeks PI. From 16 weeks PI, egg excretion was very low in all three dose groups. Liver tissue egg counts increased significantly between 4 and 11 weeks PI only in the 2000 cercariae dose group, and tended to decrease thereafter. The number of viable worm pairs decreased significantly after 11 weeks PI in the 2000 cercariae dose group, but not in the other groups. There was a strong correlation between the number of viable worm pairs and egg counts in faeces and in the liver, respectively, at 11 weeks PI. Liver and faecal egg counts were also correlated at that timepoint. The only haematological abnormality was eosinophilia with a peak corresponding to the peak in egg excretion in all infected pigs, although only the 2000 dose group differed significantly from the controls.

Gross pathology (Papers I and II)

Gross lesions were predominantly found in the large intestine and liver, and were most severe in the 2000 dose group at 11 weeks PI, after which they gradually decreased. Intestinal lesions were characterised by multifocal aggregates of hyperaemic foci and petechial haemorrhages. Sometimes echymotic and more extensive haemorrhages occurred, and the mucosa was occasionally ulcerated. In the 2000 dose group at 17 weeks PI and in all dose groups at 24 weeks PI, numerous small nodules with dead worms or worm remnants were detected in the mesenteric vasculature throughout the large bowel. Liver lesions were typically disseminated small whitish nodules and multifocal or generalised fibrosis. The degree of fibrosis was proportional to the initial cercarial dose, and tended to correlate with liver tissue egg counts. Enlargement of portal lymph nodes was common, and ascites was seen sporadically. Small intestinal lesions were mild multifocal hyperaemia and haemorrhages, and occasional worm nodules, and were found mainly in the 2000 dose group. Occasional small firm nodules were detected in the lungs. No gross lesions were found in other organs.

Histopathology (Paper II)

Egg-related lesions occurred mainly in the large intestine and liver, and were detected from 11 weeks PI. In the 2000 dose group, eggs and lesions were abundant at 11 weeks PI and then gradually decreased with increased duration of infection, whereas the 100 dose group consistently showed mild lesions. In the intestine, most eggs were in the mucosa and free of tissue reaction. Mucosal

lesions were typically acute exudative inflammatory foci comprised of recently laid egg clusters, eosinophils, macrophages, giant cells, and small mononuclear cells. These foci frequently caused intestinal crypt dilations and disruption of crypt epithelium, leading to expulsion of eggs and inflammatory cells. Granulomas of different developmental stages and containing intact or degenerated eggs were predominant in the submucosa. Early exudative-productive stage granulomas were large and showed a central accumulation of mostly degenerated eosinophils, surrounded by loosely organised macrophages, epithelioid cells and giant cells, and a peripheral zone of eosinophils and small mononuclear cells. The more mature productive stage granulomas were of variable size and were dominated by well-organised epithelioid cells and giant cells in the centre, and infiltration of inflammatory cells at the periphery. Late involutional granulomas were small and consisted of epithelioid cells and/or giant cells and showed only slight infiltration of eosinophils and small mononuclear cells. There was a nonsignificant tendency for involutional stage granulomas to increase in relative frequency with prolonged duration. Prominent concentric fibrosis, present in productive and involutional stage granulomas, was uncommon at 11 and 17 weeks PI, but increased significantly at 24 weeks PI.

In the liver, most eggs were enclosed in productive and involutional stage granulomas at all timepoints. Intact eggs were rare, and fibrotic granulomas were common already at 11 weeks PI, with a marked tendency to increase further in relative frequency at the later stages of infection. Granulomas were regularly found in portal triads and in interlobular septa. Portal venules showed variable degrees of endophlebitis, phlebitis and periphlebitis, and were frequently completely obstructed by granulomas There was a generalised periportal and septal hepatitis with infiltration of eosinophils and mononuclear cells, and periportal and septal fibrosis. Some larger portal venous branches showed marked perivascular fibrosis, but usually only slight inflammation, and eggs were rarely present. The structure of the hepatic parenchyma was essentially not affected. Liver granuloma density was significantly higher in the 2000 dose group than in the 100 dose group at all time-points, and lower at 24 compared to 11 weeks PI within the 2000 dose group. The total hepatic fibrosis scores were significantly higher in the 2000 dose group than in the 100 dose group at all three timepoints, and lower at 17 and 24 compared to 11 weeks PI in both dose groups. The total fibrosis scores were correlated to granuloma density at all three timepoints, and to EPG liver at 11 and 24 weeks PI.

Tissue reactions to worms in veins of the intestinal wall and mesentery were first noted at 11 weeks PI, and then became more prevalent at the later stages. Characteristic lesions were endophlebitis and periphlebitis, thrombophlebitis, and large granulomas with worm remnants and black schistosomal pigment. Worm granulomas were occasionally found in the liver as well. Scattered or clustered egg granulomas were detected in the lungs of a few pigs in the 2000 dose group at

each of the three later timepoints. Occasional granulomas were noted in mesenteric and portal lymph nodes and in the stomach in a few of the pigs.

Immunomorphology of the hepatic egg granuloma (Paper III)

In the early stage of infection (9 weeks PI), only large vigorous exudative-productive and productive stage granulomas were present. At 12 weeks PI, most granulomas were of these two stages, but a minor proportion of involutional stage granulomas was also found. At these two timepoints about a third of the granulomas displayed viable eggs. In the single-infected pigs at 21 weeks PI, only productive and involutional stages were present and viable eggs were few, whereas the primary and challenge-infected pigs killed at that timepoint, in comparison, showed a small proportion of exudative-productive stage granulomas, a higher frequency of granulomas with viable eggs, and a higher granuloma density. Eosinophils constituted about 30-50% of the granuloma cells in exudative-productive stage, 20% in productive stage, and 10-20% in involutional stage granulomas, with no difference observed in relation to duration of infection.

Expression of MHC class II antigen was detected in epithelioid macrophages in the central zone and in scattered cells in the peripheral zone of all granuloma stages, and was similar at the different timepoints. All granulomas also contained $CD3^+$ T cells with proportions decreasing from 40-60% in the exudative-productive stage to 20-30% in the involutional stage. Both $CD4^+$ and $CD8^+$ subsets were present, as a rule with a ratio of the average proportions (CD4:CD8) of 0.5, although it was lower (0.2) in involutional granulomas at 21 weeks PI in the single-infected pigs. Occasional γδ T cells were detected, especially at 9 weeks PI. T cells were mainly located in the peripheral zone of the granulomas. B cells expressing the CD21 antigen were prominent in small lymphoid nodules located eccentrically at the granuloma border, but were rare within the granulomas themselves. Neither granuloma-associated nodules nor granulomas showed cells positive for surface-IgM, but diffusely distributed IgG^+ plasma cells were detected in the majority of the granulomas. The proportion of these was about 10% in exudative and productive granulomas, whereas involutional stage granulomas showed only a few IgG^+ cells. Cells with cytoplasmic IgA and IgM were infrequently found and only in low numbers. About 10% of the cells, mainly lymphocytes, in the peripheral zone of exudative-productive and productive stage granulomas expressed the IL-2 receptor (CD25), indicating cell activation. Expression of CD25 was low or undetectable in involutional stage granulomas.

Naturally acquired infection (Paper IV)

Serology, parasitology and clinical signs

All group A pigs were seropositive for *S. japonicum* in both the IHA test and the ELISA on all 4 days they were checked, with low antibody levels on day 1 and 34, and a marked increase thereafter. Specific antibodies, at variable levels, were

only detected from day 82 in group B. The group A pigs were first found positive on faecal examination using the miracidial hatching test on day 34, and the group B pigs on day 82. Both groups remained positive until the last faecal samples were collected, on day 154. The weight gain of group B was reduced with 47% as compared to that of the uninfected controls, and a lesser reduction (16%) was noted also in group A. Clinical signs of illness, including fever (40-41°), diarrhoea and loss of appetite, were observed in five group B pigs on day 82. The mean liver tissue egg counts were similar in both groups: 372 EPG in group A and 336 EPG in group B, but individual variation was considerable, with ranges of 16-1100 EPG in group A and 24-820 EPG in group B. All pigs harboured *Balantidium coli* in the intestines. Thirteen pigs (nine exposed and all four controls) had *Ascaris* infection and six pigs (five exposed and one control) were infected with *Trichuris suis*.

Gross pathology

Gross lesions were in general mild or moderate, and were similar in the two groups. The caecum, colon and rectum showed multifocal mucosal lesions, either small hyperaemic foci and petechiae, or mucosal thickenings with a finely granular surface. Intact worms were frequently found in the mesenteric vasculature, as were slightly thickened vein segments with black schistosomal pigment, indicating dead worms. The interlobular network pattern of the liver was generally increased, and there were disseminated small whitish nodules and starshaped scars on the surface, and small nodules on the cut surface. In addition, 'milk spots' typical of *Ascaris* larval migration occurred in some of the pigs. Schistosome worms were rarely found in the liver. Portal lymph nodes were often enlarged. Occasional findings were slight splenomegaly, mild ascites, and small pulmonary nodules.

Histopathology

Schistosome eggs and / or lesions related to them were regularly found in the intestines and liver, and sporadically in lungs, portal lymph nodes, spleen and pancreas. The histopathology of the intestinal mucosa was characterised by numerous clusters of immature and mature eggs, often viable-appearing, and without focal inflammatory reaction, and acute inflammatory foci with egg clusters, eosinophils, macrophages and giant cells. These foci were frequently associated with discontinuities of the crypt epithelium, releasing eggs and inflammatory cells to the gut lumen. In the submucosa, eggs were enclosed in superficially located granulomas surrounded by a diffuse infiltrate of eosinophils and macrophages. Granuloma numbers were usually low, but occasionally large clusters of granulomas associated with marked submucosal fibrosis were present.

Liver lesions were characterised by granulomas in portal triads and interlobular septa, frequently causing obstruction, and sometimes obliteration, of portal veins and venules. Mean granuloma density was 1.4 (range 0.8-1.9) for group A and 1.5 (range 0.7-2.5) for group B. Scattered viable or dead eggs without tissue reaction

were also present. There was mild or moderate diffuse interstitial hepatitis, bile duct hyperplasia, periportal and septal fibrosis, and granulomatous endophlebitis, phlebitis and fibrosis of some larger portal veins. Black schistosomal pigment commonly occurred in macrophages at the periphery of granulomas. Typical lesions caused by *Ascaris* larval migration were also frequently present.

Several differences between the intestinal and hepatic granulomatous responses were found. Most intestinal granulomas were vigorous (exudative-productive and productive stages), compared to only a minority of those in the liver. Fiftytwo percent of the intestinal granulomas contained intact eggs compared to 33% of those in the liver. Intestinal granulomas showed mainly eosinophil infiltration, whereas lymphocytes predominated in the liver. However, productive and involutional stage granulomas were frequently fibrotic in both organs. Another organ-related difference became apparent when granulomas containing intact, mature eggs were analysed irrespective of developmental stage. In the intestine the majority of these granulomas (82%) were vigorous compared to only 36% of the liver granulomas. Most (64%) of the liver granulomas with intact, mature eggs lacked significant inflammatory cell infiltration, and thus appeared to be modulated.

Exudative thrombophlebitis or granulomatous endo- and periphlebitis with luminal obstruction and a marked thickening of the vessel wall were characteristic lesions related to worms in the intestinal and mesenteric vasculature.

General discussion

Method of experimental infection

Natural infection with *Schistosoma japonicum* occurs via cercarial penetration of the skin or the mucosa of the upper alimentary tract [114]. In the experiments of this thesis, however, the pigs were infected via intramuscular injection of medium-suspended cercariae, and thus the skin-penetrating stage was bypassed. This method was found to result in higher levels of worm establishment and total egg production than percutaneous methods in an earlier study in pigs [165]. Intramuscular injection of cercariae also has advantages in terms of practicality and safety. Medium-suspended cercariae lose their ability to penetrate skin [115], whereby accidental infection of humans is avoided. The rapidity of injection compared to percutaneous penetration makes it possible to infect a large number of animals in a limited amount of time, which is of value considering the short life span of cercariae. The dose of infection is also easier to standardize. However, the possible consequences of the intramuscular route on the immunological response are not known, but the number of eggs per female worm recovered from faeces and liver tissue was similar following percutaneous and intramuscular infection in the above study [165]. The intramuscular route thus did not appear to significantly influence the fecundity of the worms, a feature of the population dynamics of the parasite that is known to be affected by the immune response of the host [107].

Pathology of the intestine

In both the experimentally and the naturally *S. japonicum*-infected pigs, pathological lesions occurred multifocally in the large intestine (papers I, II and IV). The multifocal or segmental distribution of intestinal lesions due to *S. japonicum*, described in several species, is related to the tendency of worms to aggregate and remain in the same location for long periods or repeatedly return to a few specific sites for egg deposition [29]. In commonly used small laboratory animals, e.g. mice, lesions tend to be concentrated in the small intestine, whereas in large animals and man, the large bowel is primarily affected [39, 67, 89]. In humans this may be a result of a preference by the worms to reside in the inferior mesenteric vein and the superior haemorrhoidal vein [39]. The present results indicate similar worm behaviour in pigs.

The gross lesions detected in the pigs were hyperaemic foci, petechiae, mucosal thickenings with a finely granular surface, and occasionally ulcerations, which were all the result of ongoing egg excretion. Microscopically, the superficial submucosa showed egg granulomas associated with diffuse inflammatory cell infiltration, and sporadically in the naturally infected pigs (paper IV), aggregations of granulomas were associated with submucosal fibrosis. It is difficult to compare these findings to those of human schistosomiasis japonica, since most pathological descriptions deal with severe changes of longstanding,

chronic disease [35, 37, 89]. However, a few reports of acute lesions, based on proctoscopy or necropsy, describe patchy hyperaemia, ulcerations, mucosal granularity, and yellowish miliary nodules [63, 67, 122, 147]. The acute phase may last from a week to several months in humans and is frequently associated with diarrhoea or dysentery [38, 122]. The present findings thus seem similar to those of the acute phase of human disease.

Pathology of the liver

The most prominent lesion in chronic human schistosomiasis japonica, pipestem fibrosis of the liver, is characterised by thick sleeves of fibrotic tissue surrounding the intrahepatic branches of the portal vein [144]. In several animal species, *S. japonicum* infection is associated with some degree of hepatic fibrosis, but only chimpanzees develop characteristic pipestem lesions [29, 93]. Rabbits show marked periportal fibrosis resembling pipestem fibrosis combined with cirrhotic changes [26, 149]. In both chimpanzees and rabbits, the lesions develop rapidly (2-3 months after infection). Mice infected with *S. mansoni* , but not with *S. japonicum*, may also develop pipestem fibrosis in long-term mild infections [7]. Previous studies in pigs have shown variable results. Marked hepatic fibrosis was reported in pigs experimentally infected with a Chinese *S. japonicum* strain, and in naturally infected pigs in the Philippines [51, 81], but it is not clear from either of those studies whether the lesions were similar to pipestem fibrosis. In other studies of pigs, no significant hepatic fibrosis was recorded [73, 130, 176].

The experimentally infected pigs in papers I and II showed hepatic fibrosis, proportional in degree to the initial cercarial dose. Typical pipestem lesions were not observed grossly, but several characteristic microscopic features were present, i.e. egg granulomas in portal areas, portal inflammatory cell infiltration and fibrosis, destructive lesions of portal radicles, and conservation of hepatic lobular structure [92]. In addition to periportal fibrosis, inflammation and fibrosis of interlobular septa were observed in the pigs. Septal fibrosis, i.e. broad bands of fibrous tissue connecting portal triads, is a typical lesion in humans and rabbits, and within these septa there are usually large numbers of calcified eggs [2, 27, 118, 148, 149]. In contrast, eggs and granulomas were not common in the fibrotic septa of the pigs, perhaps suggesting a different pathogenesis from that in rabbits and humans. The observed pattern of septal fibrosis in pigs has similarities with diffuse schistosomal fibrosis, infrequently reported in human schistosomiasis mansoni [13, 65].

The hepatic fibrosis observed (papers I and II) was most pronounced at 11 weeks PI, and then regressed at the later stages, with the most severe lesions and the most dramatic regression seen in the 2000 cercariae dose group. The degree of fibrosis, as assessed microscopically, was correlated with egg and granuloma density in the liver in the acute as well as in the chronic stage. The regression of fibrosis was coincident with the death of most of the worms, and ceased egg production, and both these factors may have contributed to the regression. At 11

weeks PI, liver tissue egg density was correlated with both faecal egg counts and the number of viable worm pairs. These results indicate that in the acute phase of infection, faecal egg counts can be assumed to reflect the infection intensity as well as the degree of hepatic fibrosis in pigs. A similar correlation between the number of faecal and hepatic eggs, and the presence of fibrosis in liver biopsies, was found in an Indonesian study of 52 humans infected with *S. japonicum* [88]. A relationship between hepatic fibrosis and intensity of infection has also been demonstrated in rabbits, in which liver collagen content correlated well with the number of eggs in the liver as well as with the number of worm pairs [26].

Hepatic fibrosis in schistosomiasis is related to the presence of eggs and the immunological response to them by the host, but the mechanisms involved are incompletely understood [34]. In murine schistosomiasis mansoni, the development of hepatic fibrosis is closely related to granuloma formation. Most of the fibrosis is believed to be a result of scarring from confluent egg granulomas, and granuloma-associated fibrosis has been studied in detail in this model [5, 7, 152, 168]. Soluble antigens from the eggs stimulate $CD4^+$ T cells in the granulomas to secrete a variety of proinflammatory and fibrogenic lymphokines, and activated granuloma macrophages may secrete fibronectin, which is chemotactic for fibroblasts [169]. The fibrogenic events in the granuloma are most active in the early phase of infection and then decrease [110]. However, granuloma size and fibrosis appear to be regulated by partly different mechanisms [32]. A suggested pathogenesis for pipestem fibrosis, based on studies of the murine *S. mansoni* model, is that the increased portal pressure following obstruction of portal venules leads to opening of small collateral vessels along the larger portal branches, in which eggs continuously may be trapped and stimulate fibrosis, eventually resulting in typical pipestem lesions [9].

However, the relationship between granuloma scarring and periportal fibrosis in larger animal species and man is not straightforward. Andrade [3] stressed the importance of portal hepatitis for the development of pipestem fibrosis in human schistosomiasis mansoni. In *S. japonicum*-infected chimpanzees and rabbits, fibrosis around larger branches appear before eggs and granulomas are found in these sites [26, 93]. Grimaud and Borojevic [64], examining wedge liver biopsies from patients with chronic schistosomiasis mansoni, suggested that portal hypertension, subendothelial transudation of serum containing worm and egg-derived toxins and antigens, and the thereby induced inflammation and proliferation of connective tissue cells from the vascular wall, might act synergistically to produce portal fibrosis. In accordance with findings in chimpanzees and rabbits, the pigs examined in paper I and II frequently showed perivascular inflammation and fibrosis, but rarely eggs and granulomas, in larger portal veins. In addition, the amount of fibrosis in smaller portal triads and interlobular septa in general appeared to be extensive in relation to the number of granulomas. It thus seems unlikely that granuloma scarring would be the major cause of hepatic fibrosis in pigs. The fibrosis may instead mainly be related to the

perivascular and diffuse interstitial inflammation, perhaps induced by continuous low grade irritation by substances derived from eggs and worms, and various mediators arriving with the portal blood from inflammatory sites in the intestine.

Morphology of the egg granuloma and organ-related differences

The dynamics of the granulomatous reaction to eggs was investigated in papers II, III and IV. It was possible to classify the granulomas, on morphological grounds, into three developmental stages: an exudative-productive, a productive and an involutional stage (paper II). This classification system was based on the definitions by Hsü et al. [74, 75], and it was found useful for comparisons of the tissue response both between different groups of pigs, and between the liver and intestine. Comparison of the experimentally and naturally infected pigs (paper II and IV), showed that the granulomatous response to eggs in the intestine was similar, with presence of all three developmental stages in roughly comparable proportions. Granulomas with prominent fibrosis were common in the naturally infected pigs, and in the experimentally infected pigs at 24 weeks PI.

The pattern for liver granulomas was, however, more varied. In the experimental pigs of paper III, granuloma dynamics in relation to duration of infection appeared as follows: at 9 weeks PI, exclusively large, vigorous exudative-productive and productive stage granulomas were found. At 12 weeks PI, both these two stages were represented, along with some involutional stage granulomas, and at 21 weeks PI, only productive and involutional stage granulomas occurred. The proportions of granulomas with intact eggs decreased from about 30% at 9 weeks to 6% at 21 weeks PI. These findings are consistent with results of other studies in different species, which have shown a high proportion of exudative lesions in the early phase of *S. japonicum* infection, and a gradual increase in the proportions of productive and healing lesions with prolonged duration [69, 74, 93].

In the experiment of paper II, the hepatic granulomatous response did not follow the expected pattern, showing only productive and involutional stage granulomas, and very few intact eggs already at 11 weeks PI in the 2000 cercariae dose group. In this dose group, there was a prominent peak in faecal egg excretion at 8-10 weeks PI, and a significant increase in the EPG liver between 4 and 11 weeks PI, and one would thus have expected numerous newly arrived eggs in the liver inducing vigorous granulomas at 11 weeks PI. It has been suggested that eggs that remain immature or die before reaching maturity may induce productive stage granulomas directly, bypassing the exudative-productive stage [75]. One proposed mechanism for this phenomenon is antibody-mediated inhibition of egg embryonation, leading to less vigorous granuloma formation [111]. Another plausible explanation for the present observations is immunomodulation of the granulomas, i.e. a reduction of the size of granulomas formed around viable mature eggs [49]. This occurs in murine schistosomiasis japonica, where granuloma size peaks at 8-10 weeks PI, and then decreases spontaneously [121].

Possibly, the marked difference between the hepatic granulomatous response at 11 weeks PI in the pigs in paper II, and that of the pigs at 12 weeks PI in paper III could be related to infection dose (2000 cercariae in paper II and 850 cercariae in paper III), since mice heavily infected with *S. japonicum* have been shown to form smaller hepatic granulomas around eggs than lightly infected mice [31]. One could also speculate that the above mentioned inhibitory effect of antibodies on the immature eggs [111] might have been more pronounced in the high than in the low dose infections.

The pattern of hepatic granulomatous inflammation observed in the livers of the naturally infected pigs (paper IV) was similar to that in experimental infection at 21 weeks PI, except that more granulomas with viable eggs were found. The majority of granulomas with mature, viable eggs were small, involutional type, lacking significant infiltration of lymphocytes and eosinophils. It seems probable that those granulomas represented a modulated immune response to eggs. In contrast, intestinal granulomas with viable eggs in general appeared vigorous in nature, with no sign of modulation. Another apparent organ-dependent difference in the naturally infected pigs was that eosinophil infiltration dominated in vigorous, intestinal granulomas (both exudative-productive and productive stage), whereas vigorous productive stage granulomas in the liver showed mainly lymphocytic infiltration. Organ-dependent differences in the granulomatous response have been noted in other studies as well. *Schistosoma japonicum*-infected mice also showed eosinophils predominantly in granulomas of the intestine, whereas in mice infected with *S. mansoni*, hepatic granulomas were, in contrast, more eosinophil-rich than those in the intestine [72, 162].

Immunoreactivity of the hepatic egg granuloma

The results presented in paper III indicated that the hepatic egg granuloma in the pig was fundamentally an MHC class II-dependent immune reaction involving $CD4^+$ T cells. In both murine schistosomiasis mansoni and japonica, the perioval granuloma is T cell-dependent, and associated with a Th2-like cytokine pattern in the early phase of infection [33, 142, 171, 174]. The murine *S. mansoni* -induced granuloma, which by far is the most studied, is mediated by schistosome egg antigen-specific MHC class II restricted $CD4^+$ Th cells [71, 100]. The present findings thus suggest basic immunopathogenetic similarities between the porcine and the murine granuloma. The response may be Th2-dominated in the early stage of infection also in pigs, since expression of mRNA for IL-4 and IL-10, but not for IFN-γ, was found to be elevated at 10 weeks PI in intestinal and liver tissue from *S. japonicum* -infected pigs [125]. Furthermore, eosinophil infiltration was usually prominent in the present, vigorous granulomas, and the Th2-associated IL-5, which promotes eosinophil differentiation, has previously been linked to eosinophil infiltration in schistosome egg granulomas in other species [33].

Expression of both CD4 and CD8 was apparent in granulomas of the pigs. The porcine immune system differs from that in several other species in that significant numbers of extra-thymic $CD4^+CD8^+$ double-positive (DP) T cells, in addition to $CD4^+$ and $CD8^+$ single-positive (SP) cells, normally are present in peripheral blood and secondary lymphoid organs [181]. Functionally, these $CD4^+CD8^+$ DP cells are MHC class II-restricted Th cells, which express CD8 when they are activated. They are probably memory cells, since they express memory T cell markers, increase in number with the age of the pig, and produce IFN-γ [181]. It is reasonable to assume that some of the granuloma cells were $CD4^+CD8^+$ DP cells, a possibility, however, not investigated in the present thesis. A double-staining indirect immunofluorescence technique would probably be the method of choice to clarify this matter.

However, the proportion of $CD8^+$ cells was in general higher than that of $CD4^+$ cells in the granulomas, which suggests that a significant proportion of the $CD8^+$ cells were of the $CD4^-CD8^+$ SP phenotype. In pigs, this comprises MHC class I-restricted T cytotoxic/suppressor cells as well as NK cells [173]. In the murine *S. japonicum* model, T suppressor cells have been shown to down-regulate granuloma size in the acute, but not in the chronic stage of infection [121]. Both suppressor T cells and NK cells have been shown to be involved in the down-regulation of the murine *S. mansoni* granuloma [66, 128], but their importance for the down-regulation process has been questioned in other studies [71, 129, 175]. There was an increase in the proportion of $CD8^+$ cells in involutional granulomas, while the proportion of $CD4^+$ cells remained stable, in the single-infected pigs at 21 weeks PI, but not in the challenge-infected pigs at 21 weeks PI, or at the earlier timepoints. These results, though ambiguous, suggest that $CD8^+$ cells may have a role in granuloma involution, probably via suppression of immunological activity. Further investigations are clearly warranted.

B cells and their products are not necessary for granuloma formation in murine schistosomiasis japonica [30], but appear essential for immunomodulation [121]. B cells seem to have a role in the down-regulation of granulomas in murine schistosomiasis mansoni as well, since they increase in number in the modulatory phase, and granulomas in genetically B cell-deficient mice fail to undergo spontaneous modulation [58, 79].

B cell involvement in the perioval granulomas of the pig was indicated by the consistent expression of CD21 by cells in small granuloma-associated lymphoid nodules, and a significant infiltration of IgG^+ plasma cells in vigorous granulomas throughout the period of infection studied. The granuloma-associated lymphoid nodules were similar to lymphoid follicles in the control lymph node with respect to expression of CD21 and MHC class II, but differed in that cells expressing surface IgM were lacking. Surface-IgM^+ and $CD21^+$ cells were rare within the granulomas, despite the presence of numerous plasma cells. This could be explained by the fact that expression of CD21 is lost during the final

differentiation of B cells into plasma cells, and that surface IgM is down-regulated during the Ig isotype switch [46, 48]. It also indicates that B cell activity preceding the effector cell stage in these granulomas mainly took place in the associated lymphoid nodules. There appears to be no record of such events in other animal species infected with schistosomes.

One function of antibody produced within the granulomas may be to neutralize antigens emanating from the egg, thus preventing release of these antigens to the surroundings of the granuloma [15]. The locally produced antibody might also mediate cellular cytotoxicity by binding to Fc receptors on local macrophages and eosinophils [10]. The predominance of IgG-containing cells over IgA- and IgM-containing cells in granulomas at all timepoints examined in the present pigs, is different from findings in murine schistosomiasis mansoni, in which IgM-secreting cells were most common in hepatic granulomas in both acute and chronic stages [52], but similar to findings in chronic human schistosomiasis mansoni [15]. Numerous IgG-containing cells were found in hepatic granulomas in murine schistosomiasis japonica [30], but the prevalence of cells producing other isotypes seems not to have been reported in this model.

Clinical disease and effect on growth

About half of the naturally infected young pigs (group B) in the field study (paper IV) showed fever, anorexia and diarrhoea in the early egg excretion phase. Although other causes of these symptoms cannot be entirely excluded, the temporal relation to early egg excretion suggests that they were due to the *S. japonicum* infection. In contrast, the only clinical sign observed in the experimentally infected pigs of comparable age (papers I and II) was mild diarrhoea in a few pigs in each dose group in connection with initial egg excretion. Clinical signs previously reported to occur in naturally *S. japonicum*-infected pigs, especially in very young pigs, include fever, weakness, depression, anorexia, cough, nasal discharge, bloody stools, anaemia, emaciation and death [51, 179]. In older animals, the infection tends to be more chronic, and associated with distension of the abdomen and enlargement of the liver and spleen, and, in pregnant sows, with abortions and stillbirths [51, 179]. Experimentally, severe clinical disease, resembling Katayama fever in humans, has been observed in pigs after massive cercarial exposure [176], whereas no or very mild clinical signs were found in other experimental studies using low cercarial doses [81, 130].

It is thus apparent that young pigs may develop serious clinical disease from natural infection with *S. japonicum*, and that the infection can be associated with chronic illness in older pigs. The lower morbidity observed in some experimental infections could have a variety of explanations, such as the use of single cercarial exposures compared to the repeated low-level infections over a long time period presumed to occur in the field. Differences in pathogenicity between different geographic strains of *S. japonicum* could also be important, as has been shown in inbred mice [155].

It has been demonstrated both in China and the Philippines that *S. japonicum* infection in man is associated with stunted childhood growth and development [104, 105]. Stunted growth has also been reported in naturally *S. japonicum*-infected pigs and cattle, but no data on weight changes were presented [51, 67, 179]. The weight gain of the group B pigs in the present field study was reduced with 47% compared to that of the uninfected controls, and a lesser reduction (16%) was noted also for group A. Pigs reared outdoors have been shown to have a reduced daily weight gain of about 16% compared to pigs reared indoors in pens [53]. These results may thus have been influenced by the fact that the control pigs were kept in pens all the time, whereas the exposed pigs were only penned at night. This difference in management could, however, only explain part of the reduction of the weight gain in group B. Impacts of coinfections with *Trichuris suis* and *Ascaris suum* were probably only minor, since those occurred in both controls and principals. The present results thus indicate that *S. japonicum* infection naturally acquired under field conditions may markedly influence the weight gain of young pigs.

Self cure

As reported in paper I, the pigs in the experimental 2000 cercariae dose group had eliminated most of their adult worms by 24 weeks PI, as evidenced by the recovery of only a few live worms, the presence of numerous dead worms enclosed in small nodules in mesenteric veins, and ceased faecal egg excretion. This apparent ability to self cure some months after infection has been observed in experimentally infected pigs as well as in water buffaloes in other studies [178]. However, self cure was not evident in the naturally infected pigs (paper IV), despite obvious signs of worm death in the vasculature. The timing of antibody responses and faecal egg excretion for each group showed that group A had been infected for at least 26 weeks, and group B for at least 18 weeks, by the time they were killed. At necropsy, most of the pigs showed intact adult worms in their mesenteric vasculature, and the presence of numerous viable eggs in the intestinal mucosa indicated recent oviposition. In addition, all of the pigs were still excreting viable eggs about two weeks earlier.

The difference between experimental and natural infections could, again, be related to type of exposure, since repeated low level exposures had less impact on the immune response than high single doses in *S. mansoni*-infected rhesus monkeys [157]. In addition, self cure occurred more rapidly in high intensity than in low intensity infections with *S. japonicum* or *S. mansoni* in rhesus monkeys [24, 25]. However, in a Philippine study of pigs there was no spontaneous recovery 5-6 months after single infection with 5000-6000 *S. japonicum* cercariae [176]. It thus seems that the ability to self cure is not a universal feature of pigs, but may be influenced by several other factors, including the geographic strain of *S. japonicum* and the pig breed. It is clear that the porcine immune response to *S. japonicum* needs to be further investigated.

Conclusions

- Pathological lesions induced by *S. japonicum* in the pigs were essentially confined to the large intestine and liver. In the experimentally infected pigs, the liver lesions were proportional in degree to the intensity of infection.

- Gross lesions in the large intestine, including multifocal areas of hyperaemia, haemorrhages, ulcerations and mucosal granularity, resembled those occurring in acute human schistosomiasis japonica.

- Marked hepatic fibrosis developed in early patency after a single high cercarial dose and regressed spontaneously after 17 weeks of infection. Several characteristic histopathological features of schistosomal hepatic fibrosis, including granulomatous obstruction of portal venules, and periportal fibrosis, were present. Diffuse perivascular and interstitial inflammation, rather than granuloma scarring, seemed to be the major cause of fibrosis in the pigs.

- The degree of hepatic fibrosis in the experimentally infected pigs was correlated with egg and granuloma density in the liver in both the acute and chronic stage of infection. Faecal egg excretion was correlated with liver egg density in early patency, and could be used as an indicator of hepatic pathology in the acute stage of infection.

- The porcine hepatic perioval granuloma appears to be dependent on MHC class II, and involves $CD4^+$ T cells, as well as $CD8^+$ T cells, B cells and intra-lesionally produced IgG. Signs of immunomodulation of the granuloma were apparent in the liver, but not in the intestine of the naturally infected pigs, and other organ-related differences in granuloma composition were also found.

- Natural infection, but not experimental single dose infection, was associated with clinical disease and reduced weight gain in young pigs.

- Self cure was observed in the pigs infected with a single high cercarial dose, but not in the naturally infected pigs.

Suggestions for future research

The results presented in this thesis indicate that the porcine *S. japonicum* model would be convenient for studies of early fibrogenic events in the liver and the mechanisms involved in spontaneous resolution of fibrosis. It would be especially interesting to investigate the pathogenesis of periportal fibrosis lacking an obvious spatial relation with eggs and granulomas, a phenomenon possibly relevant to humans that is difficult to examine in mice. It has not yet been established whether *S. japonicum*-infected pigs develop portal hypertension, and this matter needs to be investigated. The pig could also serve as a model of acute intestinal disease in humans, an aspect of schistosomiasis that is not much studied.

Specific features of the porcine egg granuloma, such as the B cell-dominated granuloma-associated lymphoid nodules and the possible roles of $CD4^{-}CD8^{+}$ SP and $CD4^{+}CD8^{+}$ DP T cells should be further investigated. Studies of granuloma function and regulation should provide information complementary to the existing knowledge of the schistosome egg granuloma, mainly derived from murine studies.

The association of natural *S. japonicum* infection with reduced weight gain in young pigs provides a general indication that the pig model could be used to examine the effect of schistosome infection on the nutritional status of the host.

The differences observed between the experimentally and naturally infected pigs suggest that experimental studies using trickle infections, which better mimic the natural mode of exposure, would be highly relevant when further exploring the pig as a model of human schistosomiasis japonica.

References

1. Abe T and Colley DG 1984. Modulation of *Schistosoma mansoni* egg-induced granuloma formation. III. Evidence for an anti-idiotypic, I-J-positive, I-J-restricted, soluble T suppressor factor. *J Immunol 132*: 2084-2088.
2. Albertyn LE, Ganguly BS, Cooper JE and Fataar S 1990. Turtleback liver on CT and Ultrasound: *Schistosoma japonica* revisited. *Australas Radiol 34*: 78-81.
3. Andrade ZA 1965. Hepatic schistosomiasis. Morphological aspects. *Prog Liver Dis 2*: 228-242.
4. Andrade ZA and Cheever AW 1971. Alterations of the intrahepatic vasculature in hepatosplenic schistosomiasis mansoni. *Am J Trop Med Hyg 20*: 425-432.
5. Andrade ZA 1987. Pathogenesis of pipe-stem fibrosis of the liver (Experimental observation on murine schistosomiasis). *Mem Inst Oswaldo Cruz 82*: 325-334.
6. Andrade ZA, Cox TM and Cheever AW 1993. Regression of hepatic lesions after treatment of *Schistosoma mansoni* or *Schistosoma japonicum* infection in mice: A comparative study. *Am J Trop Med Hyg 49*: 1-9.
7. Andrade ZA and Cheever AW 1993. Characterization of the murine model of schistosomal hepatic periportal fibrosis ('pipe-stem' fibrosis). *Int J Exp Pathol 74*: 195-202.
8. Andrade ZA 1994. Extracellular matrix degradation in parasitic diseases. *Braz J Med Biol Res 27*: 2273-2281.
9. Andrade ZA, Silva LM and de Souza MM 1997. An experimental approach to the pathogenesis of pipe-stem fibrosis (Symmer's fibrosis of the liver). *Mem Inst Oswaldo Cruz 92*: 699-706.
10. de Andres B, Rakasz E, Hagen M, McCormik ML, Mueller AL, Elliot D, Metwali A, Sandor M, Britigan BE, Weinstock JV and Lynch RG 1997. Lack of Fc-epsilon receptors on murine eosinophils: implications for the functional significance of elevated IgE and eosinophils in parasitic infections. *Blood 89*: 3826-3836.
11. Basch PF 1991. Schistosomes: Development, reproduction and host relations. *Oxford University Press, New York, Oxford.*
12. Bell DR 1963. A new method for counting *Schistosoma Mansoni* eggs in faeces with special reference to therapeutic trials. *Bull WHO 29*: 525-530.
13. Bogliolo, L 1957. The anatomical picture of the liver in hepato-splenic schistosomiasis mansoni. *Ann Trop Med Parasitol 51*: 1-14.
14. Bracken MM, Bailey WR and Thomas HM 1948. The lesions of schistosomiasis japonica. *Am J Pathol 24*: 611-624.
15. Brito T, Shimizu SH, Yamashiro E and Da Silva LC 1985. Chronic human schistosomiasis mansoni – schistosomal antigen, immunoglobulin and complement C3 detection in the liver. *Liver 5*: 64-70.
16. Butterworth AE 1993. Immunology of schistosomiasis. In: Jordan P, Webbe G, Sturrock RF (eds): *Human schistosomiasis. CAB International, Wallingford, Oxon, UK,* pp. 331-366.

17. Cai W, Qui D and Hatz C 1992. Studies on ultrasonographic diagnosis of schistosomiasis japonica in China - a review of selected Chinese studies. *Acta Tropica 51*: 37-43.

18. Cai W, Chen Z, Chen F, Zhou C, Liu R and Wang J 1997. Changes of ultrasonography and two serum biochemical indices for hepatic fibrosis in schistosomiasis japonica patients one year after praziquantel treatment. *Chin Med J 110*: 797-800.

19. Carney WP, Hadidjaja P, Davis GM, Clarke MD, Djajasamita M and Nalim S 1973. Oncomelania hupensis from the schistosomiasis focus in Central Sulawesi (Celebes), Indonesia. *J Parasitol 59*: 210-211.

20. Carney WP and Van Peenen PFD 1978. A mammalian reservoir of *Schistosoma japonicum* in the Napu Valley, Central Sulawesi, Indonesia. *J Parasitol 64*: 1138-1139.

21. Carroll SM, Karthigasu KT and Grove DI 1981. Serodiagnosis of human strongyloidiasis by an enzyme-linked immunosorbent assay. *Trans R Soc Trop Med Hyg 75*: 706-709.

22. Catto J 1905. *Schistosoma Cattoi*, a new blood fluke of man. *Br Med J 1*:11-13.

23. Cheever AW 1969. Quantitative comparison of the intensity of *Schistosoma mansoni* infection in man and experimental animals. *Trans R Soc Trop Med Hyg 63*: 781-795.

24. Cheever AW and Powers KG 1972. *Schistosoma mansoni* infection in rhesus monkeys: comparison of the course of heavy and light infections. *Bull WHO 46*: 301-309.

25. Cheever AW, Erickson DG, Sadun EH and von Lichtenberg F 1974. *Schistosoma japonicum* infection in monkeys and baboons: Parasitological and pathological findings. *Am J Trop Med Hyg 23*: 51-64.

26. Cheever AW, Duvall RH, Minker RG and Nash TE 1980a. Hepatic fibrosis in rabbits infected with Japanese and Philippine strains of *Schistosoma japonicum. Am J Trop Med Hyg 29*: 1327-1339.

27. Cheever AW, Duvall RH and Minker RG 1980b. Extrahepatic pathology in rabbits infected with Japanese and Philippine strains of *Schistosoma japonicum*, and the relation of intestinal lesions to passage of eggs in the faeces. *Am J Trop Med Hyg 29*: 1316-1326.

28. Cheever AW and Duvall RH 1982. *Schistosoma japonicum:* migration of adult worm pairs within the mesenteric veins of mice. *Trans R Soc Trop Med Hyg 76*: 641-645.

29. Cheever AW 1985. *Schistosoma japonicum*: The pathology of experimental infection. *Exp Parasitol 59*: 1-11.

30. Cheever AW, Byram JE, Hieny S, von Lichtenberg F, Lunde MN and Sher A 1985. Immunopathology of *Schistosoma japonicum* and *S. mansoni* infection in B cell depleted mice. *Parasite Immunol 7*: 399-413.

31. Cheever AW 1986. The intensity of experimental schistosome infections modulates hepatic pathology. *Am J Trop Med Hyg 35*: 124-133.

32. Cheever AW 1997. Differential regulation of granuloma size and hepatic fibrosis in schistosome infections. *Mem Inst Oswaldo Cruz 92*: 689-692.

33. Cheever AW and Yap GS 1997. Immunologic basis of disease and disease regulation in schistosomiasis. *Chem Immunol 66*: 159-176.

34. Cheever AW, Jankovic D, Yap GS, Kullberg MC, Sher A and Wynn TA 1998. Role of cytokines in the formation and downregulation of hepatic circumoval granulomas and hepatic fibrosis in *Schistosoma mansoni* -infected mice. *Mem Inst Oswaldo Cruz 93, Suppl I*: 25-32.

35. Chen MC, Wang SC, Chang PY, Chuang CY, Chen YJ, Tang YC and Chou SC 1978. Granulomatous disease of the large intestine secondary to schistosome infestation. A study of 229 cases. *Chin Med J 4*: 371-378.

36. Chen MG 1985. Chemotherapy of schistosomiasis japonica in China. *South East Asian J Trop Med Public Health 16*: 473-479.

37. Chen MG and Mott KE 1988. Progress in assessment of morbidity due to *Schistosoma japonicum* infection. *Trop Dis Bull 85*: R1-R45.

38. Chen MG and Fu S 1989. Schistosomiasis japonica. In: R. Goldsmith and D. Heineman (eds.): *Tropical Medicine and Parasitology. Appleton and Lange, Norwalk*, pp. 452-456.

39. Chen MG 1991. Relative distribution of *Schistosoma japonicum* eggs in the intestine of man: a subject of inconsistency. *Acta Tropica 48*: 163-171.

40. Chen MG 1993. *Schistosoma japonicum* and *S. japonicum* -like infections: Epidemiology, clinical and pathological aspects. In: Jordan P, Webbe G, Sturrock RF (eds): *Human schistosomiasis. CAB International, Wallingford, Oxon, UK*, pp. 237-270.

41. Chen MG 1995. Control and research on schistosomiasis in China. *Chin Med J 108*: 643-646.

42. Chen MG and Feng Z 1999. Schistosomiasis control in China. *Parasitol Int 48*: 11-19.

43. Cheng TH 1971. Schistosomiasis in mainland China. A review of research and control programs since 1949. *Am J Trop Med Hyg 20*: 26-53.

44. Cheng TO 1984. Glimpses of the past from the recently unearthed ancient corpses in China. *Ann Intern Med 101*: 714-715.

45. Cioli D 1998. Chemotherapy of schistosomiasis: An update. *Parasitol Today 14*: 418-422.

46. Clark EA and Ledbetter JA 1989. Structure, function and genetics of human B cell-associated surface molecules. *Adv in Cancer Res 52*: 82-149.

47. Davis A 1993. Antischistosomal drugs and clinical practice. In: Jordan P, Webbe G, Sturrock RF (eds.): *Human schistosomiasis. CAB International, Wallingford, Oxon, UK*, pp. 367-404.

48. Denham S, Shimizu M, Bianchi AT, Zwart RJ, Carr MM and Parkhouse RM 1994. Monoclonal antibodies recognising differentiation antigens on porcine B cells. *Vet Immunol Immunopathol 43*: 259-267.

49. Domingo EO and Warren KS 1968. Endogenous desensitization: Changing host granulomatous response to schistosome eggs at different stages of infection with *Schistosoma mansoni. Am J Pathol 52*: 369-377.

50. Dumag PU 1979. Animal schistosomiasis: A review. *Philippine J Anim Industry 34*: 50-64.

51. Dumag PU, Gajudo CE, Sefia CY, Cardenas EC and Fementira EB 1980. Epidemiology of animal schistosomiasis in the Philippines. *Philippine J Anim Industry 35*: 1-23.

52. El-Cheikh MC, Dutra HS, Minóprio P and Borojevic R 1994. Increase of B-lymphocyte number and activity during experimental murine schistosomiasis mansoni. *Braz J Med Biol Res 27*: 1605- 1617.

53. Enfält AC, Lundström K, Hansson I, Lundeheim N and Nyström PE 1996. Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. *Meat Science 45*: 1-15.

54. Farshy DC and Kagan IG 1973. Use of stable sensitized cells in an improved indirect microhemagglutination test for malaria. *Infect Immun 7*: 680-682.

55. Faust EC and Meleney HE 1924. Studies on schistosomiasis japonica. *Am J Hyg Monogr Ser 3*: 1-339.

56. Faust EC and Ingalls JW 1946. The diagnosis of schistosomiasis japonica III. Technics for the recovery of the eggs of *Schistosoma japonicum. Am J Trop Med 26*: 559-584.

57. Fernandez TJ Jr, Petilla T and Banez B 1982. An epidemiological study on *Schistosoma japonicum* in domestic animals in Leyte, Philippines. *Southeast Asian J Trop Med Public Health, 13*: 575-579.

58. Ferru I, Roye O, Delacre M, Auriault C and Wolowczuk 1998. Infection of B-cell-deficient mice by the parasite *Schistosoma mansoni*: Demonstration of the participation of B cells in granuloma modulation. *Scand J Immunol 48*: 233-240.

59. Frandson RD and Spurgeon TL 1992. Anatomy and physiology of farm animals. *Philadelphia, Lea & Febiger*, p. 526.

60. Fujii D 1847. An account of a journey to Katayama. *Chugai Iji Shinpo, No. 691*: 55-56 1909. Translated from Japanese. In: Kean BH, Mott KE and Russell AJ (eds.): *Tropical Medicine and Parasitology. Classic Investigations. Vol II 1978, Cornell Univ Press, Ithaca, London*, pp. 513-514.

61. Fujinami K and Nakamura H 1909. Katayama disease in Hiroshima prefecture. *Kyoto Igaku Zasshi, 6*: 224-252 (extracted). Translated from Japanese. In: Kean BH, Mott KE and Russell AJ (eds): *Tropical Medicine and Parasitology. Classic Investigations. Vol II 1978, Cornell Univ Press, Ithaca, London*, pp. 531-537.

62. Garcia EG 1976a. The biology of *Schistosoma japonicum*, Philippine strain: a review. *Southeast Asian J Trop Med Public Health 7*: 190-196.

63. Garcia EG 1976b. Clinical studies on schistosomiasis japonica in the Philippines: A review. *Southeast Asian J Trop Med Public Health 7*: 247-256.

64. Grimaud JA and Borojevic R 1986. Portal fibrosis: intrahepatic portal vein pathology in chronic human schistosomiasis mansoni. *Submicrosc Cytol 18* : 783-793.

65. Hashem M 1947. The aetiology and pathogenesis of the endemic form of hepatosplenomegaly "Egyptian splenomegaly". *J R Egypt Med Assoc 30*: 48-79.

66. Hashimoto A, Pincelli C, Fujioka A, Fukuyama K and Epstein WL 1990. Relationship between NK cells and granulomatous infection in mice. *J Clin Lab Immunol 33*: 41-47.

67. He YC and Zhu DW 1988. Schistosomiasis japonica. In: Yu E and Xu B (eds.): *Chinese Zoonoses. Fujiang Science and Technology Press, Fuzhou*: 634-647. (In Chinese).

68. He YX, Xu ST, Shi FH, Wei W, Hsü SYL and Hsü HF 1992a. Comparative studies on infections of *Schistosoma japonicum* in Bos taurus and Bubalus bubalis. *Acta Zool Sinica 38*: 266-270. (In Chinese).

69. He,YX, Yu QF, and Hu YQ 1992b. Parasitological and histopathological studies on rhesus monkeys infected with Chinese mainland strain of *Schistosoma japonicum. Southeast Asian J Trop Med Public Health 23*: 254-260.

70. He YX 1993. Biology of *Schistosoma japonicum*. From cercaria penetrating into host skin to producing egg. *Chin Med J 106*: 576-583.

71. Hernandez HJ, Wang Y, Tzellas N and Stadecker MJ 1997. Expression of class II, but not class I, major histocompatibility complx molecules is required for granuloma formation in infection with *Schistosoma mansoni. Eur J Immunol 27*: 1170-1176.

72. Hirata M, Takushima M, Kage M and Fukuma T 1993. Comparative analysis of hepatic, pulmonary, and intestinal granuloma formation around freshly laid *Schistosoma japonicum* eggs in mice. *Parasitol Res 79*: 316-321.

73. Ho YH 1963. On the host specificity of *Schistosoma japonicum. Chin Med J 82*: 403-414.

74. Hsü HF, Davis JR and Hsü SYL 1969. Histopathological lesions of rhesus monkeys and chimpanzees infected with *Schistosoma japonicum. Z Tropenmed Parasitol 20*: 184-205.

75. Hsü SYL, Hsü HF, Davis JR and Lust GL 1972. Comparative studies on the lesions caused by eggs of *Schistosoma japonicum* and *Schistosoma mansoni* in livers of albino mice and rhesus monkeys. *Ann Trop Med Parasitol 66*: 89-97.

76. Hsü SYL, Xu ST, , He YX, Shi FH, Shen W, Hsü HF, Osborne JW and Clarke WR 1984. Vaccination of bovines against schistosomiasis japonica with highly irradiated schistosomula in China. *Am J Trop Med Hyg 33*: 891-898.

77. Icatlo Jr FC, Fevidal Jr P and Olveda RM 1983. A survey of canine schistosomiasis japonica in Macanip village, Leyte province, using serum circumoval precipitin test. *Philippine J Vet Med 22*: 87-94.

78. Ishii A, Matsouka H, Aji T, Ohta N, Arimoto S, Wataya Y and Hayatsu H 1994. Parasite infection and cancer: With special emphasis on *Schistosoma japonicum* infections (Trematoda). *Mutation Res 305*: 273-281.

79. Jankovic D, Cheever AW, Kullberg MC, Wynn TA, Yap G, Caspar P, Lewis FA, Clynes R, Ravetch JV and Sher A 1998. CD4+ cellmediated granulomatous pathology in schistosomiasis is downregulated by a B-cell-dependent mechanism requiring Fc receptor signalling. *J Exp Med 187*: 619-629.

80. Jiang Q, Zhang S, Yuan H, Liu Z, Zhao G and Brinkmann U 1996. The effect of a combined approach to schistosomiasis control on the transmission of *Schistosoma japonicum* in Xingzi of Poyang Lake Area, China. *Southeast Asian J Trop Med Public Health 27*: 535-541.

81. Johansen MV 1998. Effect of praziquantel treatment on experimental porcine *Schistosoma japonicum* infection. *Parasitology 116*: 519-524.

82. Kane CA and Most H 1948. Schistosomiasis of the central nervous system. *Arch Neurol Psychiatr 59*: 141-183.

83. Katsurada, F 1904. The etiology of a parasitic disease. *Iji Shimbun, No. 669*: 1325-1332. Translated from Japanese. In: Kean BH, Mott KE and Russell AJ (eds.): *Tropical Medicine and Parasitology. Classic Investigations. Vol II 1978, Cornell University Press, Ithaca, London,* pp. 518-521.

84. Katz N, Chaves A and Pellegrino J 1972. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo 14*: 397-400.

85. Kawanishi, K 1904. A report of a study of the "Katayama disease" in Higo-No-Kuni. *Tokyo Igakkai Zasshi, 18, No. 3*: 31-48. Translated from Japanese. In: Kean BH, Mott KE and Russell AJ (eds.): *Tropical Medicine and Parasitology. Classic Investigations. Vol II 1978, Cornell University Press, Ithaca, London*, pp. 527-529.

86. Kresina TF, He Q and Zern MA 1992. Cytokines, hepatic fibrosis and schistosomiasis. *Rhode Island Med 75*: 191-195.

87. Kumar V and de Burbure G 1986. Schistosomes of animals and man in Asia. *Helminthol Abstr Ser A 55*: 469-480.

88. Kurniawan AN, Hardjawidjaja L and Clark RT 1976. A clinico-pathologic study of cases with *Schistosoma japonicum* infection in Indonesia. *Southeast Asian J Trop Med Public Health 7*: 263-269.

89. Lee RC, Chiang JH, Chou YH, Rubesin SE, Wu hp, Jeng WC, Hsu CC, Tiu CM and Chang T 1994. Intestinal schistosomiasis japonica: CT-pathologic correlation. *Radiology 193*: 539-542.

90. Li YS, Sleigh AC, Ross AGP, Williams GM, Tanner M and McManus DP 2000. Epidemiology of *Schistosoma japonicum* in China: morbidity and strategies for control in the Dongting Lake region. *Int J Parasitol 30*: 273-281

91. von Lichtenberg F 1964. Studies on granuloma formation. III. Antigen sequestration and destruction in the schistosome tubercle. *Am J Pathol 45*: 75-93.

92. von Lichtenberg F and Sadun EH 1968. Experimental production of bilharzial pipe-stem fibrosis in the chimpanzee. *Exp Parasitol 22*: 264-278.

93. von Lichtenberg F, Sadun EH, Cheever AW, Erickson DG, Johnson AJ and Boyce HW 1971. Experimental infection with *Schistosoma japonicum* in chimpanzees. *Am J Trop Med Hyg 20*: 850-893.

94. von Lichtenberg F, Sadun EH and Bruce JI 1972. Renal lesions in *Schistosoma japonicum* infected rabbits. *Trans R Soc Trop Med Hyg 66*: 505-507.

95. Logan OT 1905. A case of dysentery in Hunan province caused by the trematode, *Schistosoma japonicum. China Missionary Med J 19*: 243-245.

96. Majima, T 1888. A strange case of liver cirrhosis caused by parasitic ova. *Tokyo Igakkai Zasshi, 2; No. 17*: 898-901. Translated from Japanese. In: Kean BH, Mott KE and Russell AJ (eds.): *Tropical Medicine and Parasitology. Classic Investigations. Vol II 1978, Cornell University Press, Ithaca, London, pp.* 514-517.

97. Malek EA 1980. Schistosomiasis. *Snail-Transmitted Parasitic Diseases, Vol I. CRC Press Inc., Boca Raton, Florida, pp.* 179-307.

98. Mao SP and Shao BR 1982. Schistosomiasis control in the People's Republic of China. *Am J Trop Med Hyg 31*: 92-99.

99. Markel SF, LoVerde PT and Britt EM 1978. Prolonged latent schistosomiasis. *J Am Med Assoc 240*: 1746-1747.

100. Mathew RC and Boros DL 1986. Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. *Infect Immun 54*: 820-826.

101. Matsuda H, Tanaka H, Nogami S and Muto M 1983. Mechanism of action of praziquantel on the eggs of *Schistosoma japonicum*. *Japan J Exp Med 53*: 271-274.

102. McCully RM, Barron CN and Cheever AW 1974. Diseases caused by trematodes: Schistosomiasis. In: Binford CH and Connor DH (eds.): *Pathology of Tropical and Extraordinary Diseases, Castle House Publ., Tunbridge Wells, UK*, pp. 482-508.

103. McGarvey ST, Aligui A, Daniel BL, Peters P, Olveda R and Olds GR 1992. Child growth and schistosomiasis japonica in northeastern Leyte, the Philippines: Cross-sectional results. *Am J Trop Med Hyg 46*: 571-581.

104. McGarvey ST, Wu GL, Zhang SJ, Wang YJ, Peters P, Olds GR and Wiest PM 1993. Child growth, nutritional status, and schistosomiasis japonica in Jiangxi, People's Republic of China. *Am J Trop Med Hyg 48*: 547-553.

105. McGarvey ST, Aligui A, Graham KK, Peters P, Olds GR and Olveda R 1996. Schistosomiasis japonica and childhood nutritional status in northeastern Leyte, the Philippines: A randomized trial of praziquantel versus placebo. *Am J Trop Med Hyg 54*: 498-502.

106. McGarvey ST, Zhou XN, Willingham III AL, Feng Z and Olveda R 1999. The epidemiology and host-parasite relationship of *Schistosoma japonicum* in definitive hosts. *Parasitol Today 15*: 214-215.

107. McKerrow JH 1997. Cytokine induction and exploitation in schistosome infections. *Parasitology 115*: S105-S112.

108. McManus D 2000. A vaccine against Asian schistosomiasis: the story unfolds. *Int J Parasitol 30*: 265-271.

109. McNeely DF and Magu MR 1988. Schistosomiasis: In: McLeod CL (ed): *Parasitic Infections in Pregnancy and the Newborn, Oxford University Press, New York*, pp. 236-237.

110. Meneza SE, Olds GR, Kresina TF and Mahmoud AAF 1989. Dynamics of hepatic connective tissue matrix constituents during murine *Schistosoma mansoni* infection. *Hepatology 9*: 50-56.

111. Mitchell GF, Garcia EG, Davern KM and Tiu WU 1995. Anti-embryonation immunity in murine Schistosomiasis japonica (Philippines). *Mem Inst Oswaldo Cruz 90*: 293-295.

112. Miyáiri K and Suzuki M 1914. The intermediate host of *Schistosoma japonicum Katsurada*. (Der zwischenwirt der Scistosomum japonicum Katsurada). *Mitteilungen der Medizinischen. Fakultät Kaiserlichen Universität Kyushu, I*: 187-197 (selections). Translated from German. In: Kean BH, Mott KE and Russell AJ (eds.): *Tropical Medicine and Parasitology. Classic Investigations. Vol II 1978, Cornell University Press, Ithaca, London*, pp.540-543.

113. Miyake M 1971. Schistosomiasis japonicum. In: Marcial-Rojas, RA (ed.): *Pathology of Protozoal and Helminthic Diseases, with Clinical Correlation, Williams and Wilkins Company, Baltimore*, pp. 414-433.

114. Miyazaki I 1991. An illustrated book of helminthic zoonoses. *International Medical Foundation of Japan, Tokyo.*

115. Moloney NA and Webbe G 1982. A rapid method for the infection of laboratory mice with *Schistosoma japonicum. Trans R Soc Trop Med Hyg 76*: 200-203.

116. Moloney NA and Webbe G 1984. Factors affecting the acquisition of resistance to *Schistosoma japonicum* in the mouse. II. Evidence that resistance to reinfection is not mediated by specific effector mechanisms. *Parasitology 89*: 361-367.

117. Moloney NA, Doenhoff MJ, Webbe G and Hinchcliffe P 1982. Studies on the host-parasite relationship of *Schistosoma japonicum* in normal and immunosuppressed mice. *Parasite Immunol 4*: 431-440.

118. Monzawa S, Ohtomo K, Oba H, NogataY, Kachi K and Uchiyama G 1994. Septa in the liver of patients with chronic hepatic schistosomiasis japonica : MR appearance. *Am J Roentgenol 162*: 1347-1351.

119. Ohmae H, TanakaM, Hayashi M, Matsuzaki Y, Kurosaki Y, Blas BL, Portillo GG, Sy OS, Irie Y and Yasuraoka K 1992. Improvement of ultrasonographic and serologic changes in *Schistosoma japonicum*-infected patients after treatment with praziquantel. *Am J Trop Med Hyg 46*: 99-104.

120. Olds GR, Olveda R, Tracy JW and Mahmoud AF 1982. Adoptive transfer of modulation of granuloma formation and hepatosplenic disease in murine schistosomiasis japonica by serum from chronically infected animals. *J Immunol 128*: 1391-1393.

121. Olds GR and Stavitsky AB 1986. Mechanisms of *in vivo* modulation of granulomatous inflammation in murine schistosomiasis japonicum. *Infect Immun 52*: 513-518.

122. Olveda RM and Domingo EO 1987. Schistosomiasis japonica. *Ballière's Clin Trop Med Communicable Dis 2*: 397-417.

123. Olveda RM, Daniel BL, Ramirez BDL, Aligui GDL, Acosta LP, Fevidal P, Tiu E, de Veyra F, Peters PA, Romulo R, Domingo E, Wiest PM and Olds GR 1996. Schistosomiasis japonica in the Philippines: The long-term impact of population-based chemotherapy on infection, transmission, and morbidity. *J Infect Dis 174*: 163-172.

124. Ostrea EM and Marcelo FB 1965. Relationship between pulmonary schistosomiasis japonicum and cor pulmonale, A pathologic study. *Acta Med Philippina 2*: 68-77.

125. Oswald IP, Dozois CM, Barlagne R, Fournout S, Johansen MV and Bøgh HO. Cytokine mRNA expression in pigs infected with *Schistosoma japonicum. (Submitted for publication).*

126. Pearce EJ, Basch PF and Sher A 1986. Evidence that the reduced surface antigenicity of developing *Schistosoma mansoni* schistosomula is due to antigen shedding rather than host molecule acquisition. *Parasite Immunol 8*: 79-94.

127. Pesigan TP, Farooq M, Hairston NG, Jauregui JJ, Garcia EG, Santos AT, Santos BC and Besa AA 1958. Studies on *Schistosoma japonicum* -infection in the Philippines. *Bull WHO 18*: 345-455.

128. Ragheb S and Boros DL 1989. Characterization of granuloma T lymphocyte function from *Schistosoma mansoni*- infected mice. *J Immunol 142*: 3239-3246.

129. Rakasz E, Blum AM, Metwali A, Elliot DE, Li J, Ballas ZK, Qadir K, Lynch R and Weinstock JV 1998. Localization and regulation of IFN-gamma production within the granulomas of murine schistosomiasis in IL-4-deficient and control mice. *J Immunol 160*: 4994-4999.

130. Reid WA and von Lichtenberg F 1977. Experimental *Schistosoma japonicum* infection in miniature pigs. *J Parasitol 63*: 392-394.

131. Reyes VA, Yogore MG and Pardo LP 1964. Studies on Cerebral Schistosomiasis. *J Philippine Med Ass 40*: 87-100.

132. Rezende SA, Gollob KJ, Correa-Oliveira R and Goes AM 1998. Down modulation of MHC surface molecules on B cells by suppressive immune complexes obtained from chronic intestinal schistosomiasis patients. *Immunol Letters 62*: 67-73.

133. Robinson A, Lewert RM and Spargo BH 1982. Immune complex glomerulonephritis and amyloidosis in *Schistosoma japonicum* infected rabbits. *Trans R Soc Trop Med Hyg 76*: 214-226.

134. Ross AGP, Li YS, Sleigh AC and McManus DP 1997. Schistosomiasis control in the People's Republic of China. *Parasitol Today 13*: 152-155.

135. Ross AGP, Sleigh AC, Li Y, Davis GM, Williams GM, Jiang Z, Feng Z, Guo J and McManus DP 2000. Schistosomiasis in the People's Republic of China: Prospects and Challenges for the 21st Century. *(In press)*.

136. Shi YE, Jiang CF, Han JJ, Li YL and Ruppel A 1990. *Schistosoma japonicum*: An ultraviolet-attenuated cercarial vaccine applicable in the field for water buffaloes. *Exp Parasitol 71*: 100-106.

137. Shi YE, Jiang CF, Han YL, Li YL and Ruppel A 1993. Immunization of pigs against infection with *Schistosoma japonicum* using ultraviolet-attenuated cercariae. *Parasitology 106*: 459-462.

138. Sleigh AC and Mott KE 1986. Schistosomiasis. *Clin Trop Med Communicable Dis 1*: 643-670.

139. Sleigh A, Li X, Jackson S and Huang K 1998. Eradication of schistosomiasis in Guangxi, China. Part 1: Setting, strategies, operations, and outcomes 1953-92. *Bull WHO 76*: 361-372.

140. Sleigh A and Jackson S 1998. Public health and public choice: dammed off at China's Three Gorges? *Lancet 351*: 1449-1450.

141. Smithers SR and Terry RJ 1969. Immunity in schistosomiasis. IV. Immunological aspects. *Ann NY Acad Sci 160*: 826-840.

142. Stavitsky A B 1987. Immune regulation in schistosomiasis japonica. *Immunol Today 8*: 228-233.

143. Sturrock RF 1993. The Parasites and Their Life Cycles, The Intermediate Hosts and Host-Parasitič Relationships. In: Jordan P, Webbe G, Sturrock RF (eds.): *Human schistosomiasis. CAB International, Wallingford, Oxon, UK*, pp. 1-86.

144. Sulit YSM, Domingo EO, Dalmacio-Cruz AE, De Peralta DS and Imperial E S 1964. Parasitic cirrhosis among Filipinos. *J Philipp Med Assoc 40*: 1021-1038.

145. Tanaka H, Tsuji M, Tsutsumi H and Minai M 1984. The present status of schistosomiasis in Japan. *Southeast Asian J Trop Med Public Health 15*: 475-477.

146. Taylor MG 1994. Schistosomiasis vaccines: Farewell to the God of Plague? *J Trop Med Hyg 97*: 257-268.

147. Thomas HM, Bracken MM and Bang FB 1946. The clinical and pathological picture of early acute schistosomiasis japonica. *Trans Assoc Am Physicians 59*: 75-81.

148. Tsutsumi H and Nakashima T 1972a. Pathology of liver cirrhosis due to *Schistosoma japonicum*. In: M.Yokogawa (ed.): *Research in Filariasis and Schistosomiasis. University Park Press, Baltimore*, pp. 113-131.

149. Tsutsumi H and Nakashima T 1972b. Experimental schistosomiasis japonica in rabbits. In: M.Yokogawa (ed.): *Research in Filariasis and Schistosomiasis. University Park Press, Baltimore*, pp. 133-144.

150. Tumbleson ME and Schook LB (eds.) 1996. *Advances in swine in biomedical research. Plenum Press, New York.*

151. Visser PS and Pitchford RJ 1972. A simple apparatus for rapid recovery of helminth eggs from excreta with special reference to *Schistosoma mansoni. South African Med J 46*: 1344-1346.

152. Warren KS 1966. The pathogenesis of "clay-pipe stem cirrhosis" in mice with chronic schistosomiasis mansoni, with a note on the longevity of the schistosomes. *Am J Pathol 49*: 477-489.

153. Warren KS, Domingo EO and Cowan RBT 1967. Granuloma formation around schistosome eggs as a minifestation of delayed hypersensitivity. *Am J Pathol 51*: 735-756.

154. Warren KS and Domingo EO 1970. Granuloma formation around *Schistosoma mansoni, S. haematobium*, and *S. japonicum* eggs. *Am J Trop Med Hyg 19*: 292-304.

155. Warren KS and Berry EG 1972. Induction of hepatosplenic disease by single pairs of the Philippine, Formosan, Japanese and Chinese strains of *Schistosoma japonicum. J Infect Dis 126*: 482-491.

156. Warren KS 1973a. The pathology of schistosome infections. *Helminthol Abstr Ser A 42*: 591-633.

157. Warren KS 1973b. Regulation of the prevalence and intensity of schistosomiasis in man: Immunology or ecology? *J Infect Dis 127*: 595-609.

158. Warren KS 1979. The pathogenesis of hepatosplenic schistosomiasis: From man to monkey to mouse to molecule. *Prog Liver Dis 6*: 439-455.

159. Watt G, Long GW, Calubaquib C and Ranoa CP 1987. Prevalence of renal involvement in *Schistosoma japonicum* infection. *Trans R Soc Trop Med Hyg 82*: 339-342.

160. Watt G, Padre LP, Tuazon M, Wotherspoon A and Adapon B 1991. Hepatic parenchymal dysfunction in *Schistosoma japonicum* infection. *J Infect Dis 164*: 1186-1192.

161. Webbe G and Jordan P 1993. Control. In: Jordan P, Webbe G, Sturrock RF (eds.): *Human schistosomiasis. CAB International, Wallingford, Oxon, UK*, pp. 405-452.

162. Weinstock JV and Boros DL 1983. Organ-dependent differences in composition and function observed in hepatic and intestinal granulomas isolated from mice with schistosomiasis mansoni. *J Immunol 130*: 418-422.

163. Weinstock JV 1992. The pathogenesis of granulomatous inflammation and organ injury in schistosomiasis: Interactions between the schistosome ova and the host. *Immunol Invest 21*: 455-475.

164. Wiest PM 1996. The epidemiology of morbidity of schistosomiasis. *Parasitol Today 12*: 215-220.

165. Willingham AL, Bøgh HO, Vennervald BJ, Johansen MV, Eriksen L, Christensen NØ and Nansen P 1996. Worm establishment and egg production of *Schistosoma japonicum* in pigs infected by percutaneous methods or intramuscular injection. *Vet Parasitol 61*: 157-163.

166. World Health Organization 1993. The control of schistosomiasis: second report of the WHO Expert Committee. *WHO Tech Rep Ser 830*: 1-85.

167. Wu Z, Bu K, Yuan L, Yang G, Zhu J and Liu Q 1993. Factors contributing to reinfection with schistosomiasis japonica after treatment in the lake region of China. *Acta Tropica, 54*: 83-88.

168. Wyler DJ 1992a. Why does liver fibrosis occur in schistosomiasis? *Parasitol Today 8*: 277-279.

169. Wyler DJ 1992b. Molecular and cellular basis of hepatic fibrogenesis in experimental schistosomiasis mansoni infection. *Mem Inst Oswaldo Cruz 87, Suppl IV*: 117-125.

170. Xiao SH, Booth M and Tanner M 2000. The prophylactic effects of artemether against *Schistosoma japonicum* infections. *Parasitol Today 16*: 122-126.

171. Xu Y, Macedonia J, Sher A, Pearce E and Cheever A W 1991. Dynamic analysis of splenic Th1 and Th2 lymphocyte functions in mice infected with *Schistosoma japonicum. Infect Immun 59*: 2934-2940.

172. Xu S, Shi F, Shen W, Lin J, Wang Y, Lin B, Qian C, Ye P, Fu L, Shi Y, Wu W, Zhang Z, Zhu H and Guo W. 1993. Vaccination of bovines against Schistosomiasis japonica with cryopreserved-irradiated and freeze-thaw schistosomula. *Vet Parasitol 47*: 37-50.

173. Yang H and Parkhouse RM 1997. Differential expression of CD8 epitopes amongst porcine CD8-positive functional lymphocyte subsets. *Immunology 92*: 45-52.

174. Yang JQ, Tasaka K, Chuang CK, Yoshikawa H and Nakajima N 1999. Dynamic analysis of T-lymphocyte function in relation to hepatopathologic changes and effect of interleukin-12 treatment in mice infected with *Schistosoma japonicum. J Parasitol 85*: 257-262.

175. Yap G, Cheever A, Caspar P, Jankovic D and Sher A 1997. Unimpaired down-modulation of the hepatic granulomatous response in CD8 T-cell- and gamma interferon-deficient mice chronically infected with *Schistosoma mansoni. Infect Immun 65*: 2583-2586.

176. Yason CV and Novilla MN 1984. Clinical and pathological features of experimental *Schistosoma japonicum* infection in pigs. *Vet Parasitol 17*: 47-64.

177. Yuan HC 1993. Epidemiological features and control strategies of schistosomiasis japonica in China. *Chin Med J 106*: 563-568.

178. Zhang S, Wu Z and Lin D 1998. The phenomenon of spontaneous recovery in pigs and water buffaloes infected with *Schistosoma japonicum*. In: *Proc. Int. Symp. Schistosomosis, Wuxi, 1998*: 51-52.

179. Zhong DM and Fang HS 1981. Investigation of schistosomiasis in pigs on two breeding pig farms in Gui Chi County. *Newsletter of Parasitic Disease Treatment and Prevention, 3*. (In Chinese).

180. Zhou D, Li Y and Yang X 1994. Schistosomiasis control in China. *World Health Forum 15*: 387-389.

181. Zuckermann FA 1999. Extrathymic CD4/CD8 double positive T cells. *Vet Immunol Immunopathol 72*: 55-66.

Acknowledgements

The present study was supported by the Swedish Agency for Research Cooperation with Developing Countries (SAREC) (grant no. SWE-95-032), which is gratefully acknowledged.

Many people in Denmark, China and Sweden have contributed in various ways to this thesis, and I wish to express my sincere gratitude to all concerned. Especially I would like to thank:

Dr. Inger Haraldsson and Professor Lennart Jönsson, former and present Head of the Department of Pathology for providing laboratory and other resources, and for general support.

Associate Professor Ronny Lindberg, my main supervisor, for introducing me to schistosomiasis, for quickly and critically reviewing my many manuscript versions irrespective of their state, for support and encouragement, and for good company during our travels in the Far East.

The late Professor Peter Nansen, former head of the DCEP, and Dr. Niels Ørnbjerg Christensen, Director of DBL, my assistant supervisors, for providing the excellent research facilities that made this project possible.

Dr. Lee Willingham, my coauthor, for accepting 'pathological' modifications of your experiments, for good cooperation and friendship, and for all the shared fun during the many hours we spent in 'Sektionsstuen'.

Professor You-en Shi, my coauthor, for excellent organisation of the field study, for your hospitality during our stay in Wuhan, and for your patience with my many questions.

Dr. Adrian Sleigh, for being my link to the real 'xisto' world, for all your help and encouragement, and for your ability to make me think along new lines.

Dr. Henrik Bøgh, my coauthor, for superb practical assistance in connection with the experiments at KVL.

Dr. Maria Vang Johansen, my coauthor, for help and support, and for your kind hospitality.

Mr Niels Peter Hansen, Ms Anni Mehlsen and many others not mentioned by name at KVL and DBL for kindly and skilfully assisting me in various ways during the 137 pig necropsies.

Ms Christina Nilsson and Ms Ulla Hammarström, for innumerable beautiful tissue sections, and for your help with many other things.

Ms Briitta Ojava, for not giving up on the immunohistochemistry despite our many misfortunes, and for your kindness and conscientiousness.

Ms Anne-Sofie Lundquist, for enthusiastic assistance with countless practical matters.

Drs. Stina Ekman and Anna-Lena Berg, for friendship and encouragement over the years.

Dr. Anders Johannisson, for always helping out in computer-associated emergencies.

Drs. Khwanchai Kreausukon and Komkrich Teankum, for many interesting discussions about schisto.

All my other colleagues and friends at the Department of Pathology, for help and support in several different ways.

Mr Bengt Ekberg, for helpful hints about photography and for excellent service.

Ms Gunnel Ehrne and her staff at the SVA library, for service-mindedness and great skill in meeting my requests for exotic literature from many parts of the world.

Dr. Songhua Hu, for giving me invaluable access to Chinese literature on schisto in domestic animals via your excellent translations.

My father, for tremendous help and never failing support, and for looking after the boys during my journeys.

Martin, Magnus and Adam, my wonderful sons, for putting up with having an absent-minded mum for so long - and for being just the ones you are.

Gunnar, my love, for your total commitment.

Acta Universitatis Agriculturae Sueciae
presents doctoral theses from the faculty of agriculture, landscape architecture and horticulture, the faculty of forestry and the faculty of veterinary medicine at the Swedish University of Agricultural Sciences. Each faculty has its own subseries, Agraria, Silvestria and Veterinaria, respectively, with separate numbering.

ISSN 1401-6257
ISBN 91-576-5944-3