ELSEVIER

Veterinary Parasitology 73 (1997) 215–224

veterinary parasitology

Acquisition of resistance against *Fasciola gigantica* by Indonesian thin tail sheep

J.A. Roberts [a,1], E. Estuningsih [a], E. Wiedosari [a], T.W. Spithill [b,*]

[a] *Balai Penelitian Veteriner, Bogor, Jawa Barat, Indonesia*
[b] *Victorian Institute of Animal Science, Melbourne, Australia*

Received 7 February 1997; accepted 19 May 1997

Abstract

The plasma levels of GLDH and the dynamics of development of the numbers of surface lesions on livers, and numbers of parasites, within two to three weeks of a challenge infection, indicated that the major component of the high resistance of Indonesian thin tail (ITT) sheep against *Fasciola gigantica* was acquired, and acted against juvenile parasites. Few parasites reached the livers of ITT (exposed) sheep, but many had been inhibited, probably in the wall of the jejunum. ITT (naive) sheep showed some resistance, compared with control Merino sheep, by three weeks after infection. Manifestation of the resistance of ITT (exposed) sheep was suppressed by administration of the immunosuppressant, dexamethasone. Killing of parasites in ITT sheep appeared to have ceased by 21 to 28 days after infection. The basis of the acquired resistance was deemed to be an exceptional immunological capacity of ITT sheep responding to an antigen, or an immunological suppressant, peculiar to *F. gigantica*. That molecule, produced by juvenile parasites, warrants further study as a candidate for a vaccine. © 1997 Elsevier Science B.V.

Keywords: *Fasciola gigantica*; Indonesian thin tail sheep; Immunity; Acquired resistance; GLDH activity

1. Introduction

Indonesian thin tail (ITT) sheep have high resistance against *Fasciola gigantica* (Wiedosari and Copeman, 1990; Roberts et al., 1997a), but not against *F. hepatica* (Roberts et al., 1997a). The major mechanism of the resistance was manifest against

* Corresponding author. Department of Biochemistry and Molecular Biology, Monash University, Clayton 3168, Australia. Tel.: +61-3-99036000.
[1] Present address. Post Office Box 811, Buddina 4575, Australia.

0304-4017/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved.
PII S0304-4017(97)00119-2

immature parasites (Roberts et al., 1997a), and was controlled by a single gene with a substantial level of dominance (Roberts et al., 1997b). A less important mechanism of resistance killed some mature parasites in ITT sheep (Roberts et al., 1997a) and Merino sheep (Roberts et al., 1996) after secondary infection with *F. gigantica*. Merino sheep did not acquire resistance against *F. hepatica* (Boray, 1967).

An understanding of the mechanism of the high resistance effective against a pathogenic trematode in a mammalian host, and controlled by a single gene, could offer alternative approaches for control of *F. gigantica* and other important trematodes. It is necessary first to differentiate between innate (biochemical, physiological or anatomical), and acquired (immunological) mechanisms. In some studies of host resistance it has been possible to differentiate between innate and acquired resistance by comparing parasite yields after primary and secondary infections, as with acquired resistance of cattle against *Boophilus microplus* (Roberts, 1968), and that of sheep against *Haemonchus contortus* (Altaif and Dargie, 1978). However, the resistance of ITT sheep was manifest during primary infections (Roberts et al., 1997a), so this study determines the stage of the life cycle affected by the resistance mechanism, then compares development in exposed ITT (ITTe) with that in naive ITT (ITTn) sheep at that stage. Also, acquisition of resistance in ITTn sheep is observed by comparison with development in control naive Merino sheep. In addition, administration of the immunosuppressive drug, dexamethasone, to ITTe sheep, confirms that the resistance is immunologically based.

2. Materials and methods

2.1. Sheep

Young adult male ITT sheep were used in all experiments. In Experiments 1 and 2, ITTn sheep were purchased from the Small Ruminant-Collaborative Research Support Program at Bogor, West Java. ITTe sheep were purchased in the subdistrict of Surade, West Java, where the prevalence of *F. gigantica* is known to be high. For Experiment 3, ITT sheep were purchased in rural areas of West Java where *F. gigantica* infections occur occasionally. They were treated for gastrointestinal nematodes with 7.5 mg/kg fenbendazole, and divided on the basis of a *Fasciola* ELISA (Farrell et al., 1981) using antigen extracted from mature *F. gigantica*. The *Fasciola* ELISA was validated for *F. gigantica* by examining plasma samples after infection of naive sheep and cattle. Sheep which were clearly negative in the assay were used as ITTn, and all others were infected for use as ITTe sheep, then treated with triclabendazole at least three weeks before an experiment commenced. Merino sheep were used as a control susceptible breed in Experiments 1 and 2. They were mature wethers from Western Australia where fasciolosis does not occur. For Experiment 3, Javanese Fat Tail (JFT) sheep, purchased from the Department of Animal Husbandry Field Station, Garahan, East Java, were the control susceptible breed. Young adult males were used. The Merino and JFT sheep were naive to *F. gigantica* as determined by faecal egg counts and the ELISA. All sheep were maintained in pens on a diet of freshly cut *Pennisetum purpurium* and dairy

concentrate. Control uninfected Merino sheep were maintained under the same regimen, and plasma samples were checked from time to time with the ELISA.

2.2. Experimental design

In Experiment 1 levels of liver enzymes in plasma measured the dynamics of liver damage caused by *F. gigantica* during infection of ITTe, ITTn and naive Merino sheep. These three groups of four sheep were infected with 2000 metacercariae. From week three after infection, a control group of four naive Merino sheep, which were not infected, was included in the study. The sheep were bled weekly for plasma for 10 weeks.

The same classes of sheep were used in Experiment 2 for serial collections and measurements of parasites, and counting of lesions in livers, to determine the stage of the life cycle affected by the resistance mechanism, and to follow development of resistance in ITTn sheep. Groups of three sheep (one ITTn, one ITTe and one naive Merino), were infected with 500 metacercariae and killed at the intervals set out in Table 1. Lesions on the surfaces of livers were recorded, and parasites were counted. Only three sheep could be processed for collection of juvenile parasites in a day, so all sheep in a row of Table 1 were infected at the same time; but sheep in other rows may have been infected at different times and with different batches of metacercariae. One of the functions of the susceptible Merino sheep in each group was as a control for the viability of the metacercariae at each infection.

In Experiment 3 the effect on infection of the immunosuppressant, dexamethasone, was tested in ITTe sheep as set out in Table 2. Five groups of four ITTe sheep were infected with 200 metacercariae. Dexamethasone administration commenced to a group on each of two days before infection, and three, six and 12 days after infection. One group of ITTe sheep received no dexamethasone. A group of six naive JFT sheep acted as controls, receiving metacercariae but no dexamethasone.

2.3. Dexamethasone

The dexamethasone used was Dexafort (Intervet, Australia), which is an aqueous solution of 1 mg of rapid acting dexamethasone sodium phosphate, and a suspension of 2 mg of long acting dexamethasone phenylpropionate per ml, with eight days duration of effect. The dose rate used was 1.1 ml per 20 kg body weight, administered intramuscularly at intervals no longer than six days.

2.4. Infections with metacercariae

Metacercariae of *F. gigantica* were collected from laboratory colonies of *Lymnaea rubiginosa* infected with miracidia developed from eggs collected from the field in West Java, and from infected snails collected in the field, and so would have come from infections in both sheep and cattle. Metacercariae were stored at 14°C and used to infect when 7 to 14 days old. The cysts were gently brushed from plastic sheets in 0.001% Tween 20 in water, suspended with a magnetic stirrer, counted and divided into aliquots.

Viability of the metacercariae was determined microscopically after the outer cyst wall had been removed by pushing them through a 150 μm pore size steel mesh filter, with a rubber policeman. The main criteria for viability were clear sharp outlines of the parasites, organs and refractile granules, and movement of the parasites. Viability was better than 95%. Each aliquot was filtered onto a 15 cm diameter filter paper and the doses of metacercariae were administered orally on the filter papers with a balling gun.

2.5. Collection of parasites

Intact livers were examined, and parasite lesions on the surfaces were counted. When collecting juvenile parasites, it was not possible to cut fine slices of liver to locate and count lesions within the parenchyma because of the possibility of losing parasites, and shortage of time for processing. Before collection of parasites from livers, fat and connective tissue were trimmed, the capsule was stripped off, and the parenchymal tissue was crushed with fingers. The crushed tissue was incubated in isotonic saline at 39°C for 1 h, then separated through a filter constructed from a perforated plate 15 cm in diameter, immersed in a tray 40 × 25 cm with water to a depth of 12 cm. Separation was achieved by moving the filter gently up and down in the water so that particles which would go through the filter were separated. The filter for parasites three and four weeks old had pores 1 mm diameter, and for six weeks parasites, 2.5 mm.

The filtrate was then separated and washed with water on a nylon mesh with 75 μm pore size for parasites three and four weeks old, and 150 μm for six weeks parasites. Retention of parasites on the nylon mesh was checked by passing the filtrate through a 50 μm pore size nylon mesh and examining the residue.

The residue from the first nylon mesh was resuspended in normal saline and examined for parasites on a black tray, and in petri dishes under a stereomicroscope. The examination was completed within 24 h of collecting the parasites. Intact parasites, heads and tails were counted, and the final figure was the number of intact parasites plus the larger of the other two counts. The lengths of intact parasites were measured in normal saline against a grid under the stereomicroscope. The tissue that did not pass through the perforated plate was crushed again and the incubation, filtration and examination process was repeated until no more parasites were found.

For parasites younger than three weeks, the residues were fixed in FAA (formalin 8%, acetic acid 6% ethanol 60%) at 80°C, left for 72 h, transferred to water, and again separated as above through a series of filters with pore sizes ranging from 500 to 80 μm. The samples were kept in fixative until it was convenient to examine them.

Parasites were collected from the peritoneum by opening the abdomen of a skinned carcase along the linea alba, and removing the viscera after tying off the oesophagus and rectum. The liver was separated and the remaining abdominal viscera were immersed in a tub containing 20 l of 0.9% saline, 0.5% citric acid, 0.01% tween 20 and 0.5% formalin. The serosal surfaces were gently rubbed by hand about 10 times over a period of 2 h to remove parasites. The diaphragm, heart and lungs were removed from the carcase that was then flushed three times with 2 l of the washing fluid while gently rubbing the serosa. The fluids were sedimented and the residues were washed with saline, fixed in hot FAA and examined for parasites.

2.6. Plasma enzyme assays

Potassium EDTA was used as anticoagulant at the rate of 2 mg/ml of blood. Plasma glutamine dehydrogenase (GLDH) and plasma gamma glutamyl transferase (γGT) were measured spectrophotometrically with kits (Boehringer Mannheim Australia). The plasma for GLDH analysis was stored at -70°C and that for γGT at -20°C.

2.7. Statistical analysis

Mean values were compared by Student's t-test. A square root transformation, $\sqrt{(\text{count} + 0.5)}$, was used to normalise the parasite numbers in Experiment 3.

3. Results

No extraneous infection occurred in the negative control sheep.

3.1. Plasma enzyme levels, Experiment 1

The mean plasma GLDH values of the four groups of sheep are depicted in Fig. 1. Three and four weeks after infection, the ITTe sheep had significantly lower values

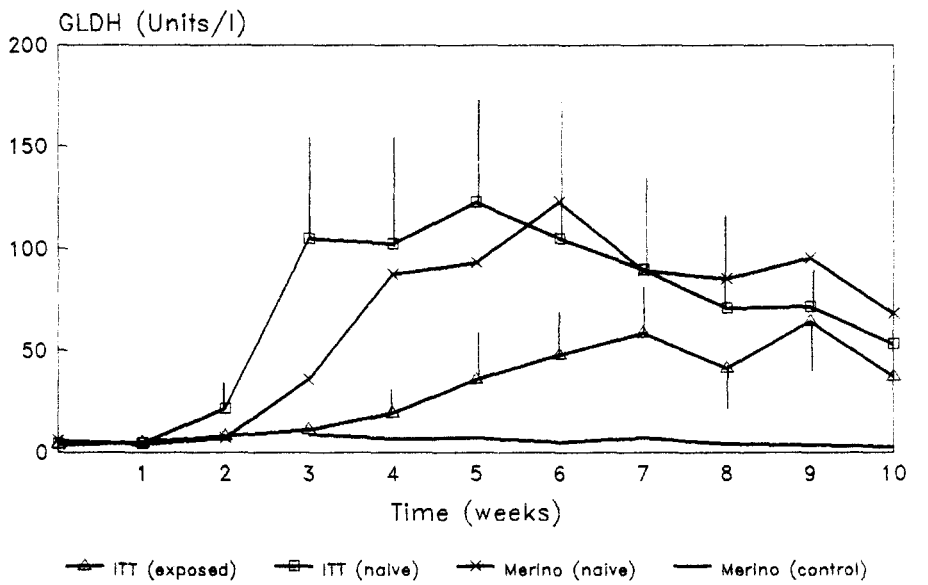

Fig. 1. Plasma glutamine dehydrogenase levels in groups of ITT (exposed), ITT (naive) and Merino (naive) sheep infected with 2000 metacercariae of *F. gigantica*. The early high levels in the naive groups indicate more extensive damage to liver parenchymal cells caused by more migrating parasites. The bars on the curves for ITT sheep represent standard deviations about the mean values.

Table 1
Numbers of parasites collected from ITT sheep after infection with 500 *F. gigantica*, and the numbers of surface lesions on the livers of the same sheep

Post-infection (days)	Sheep					
	ITT exposed		ITT naive		Merino naive	
	Parasites	Lesions	Parasites	Lesions	Parasites	Lesions
4	0	0	0	0	0	0
10	0	0	1	0	13 (2)	0
14	1	0	0	7	32	36
	1	0	2	9	43	65
	0	3	3	13	2	5
	0	0	0	1	21	41
21	nd		11	14	78	60
	nd		6	8	20	33
	0	0	0	0	48	48
	1	0	4	10	31	28
28	1	1	6	9	69	57
	1	3	4	6	37	23
42	0	2	11	11	78	63
	6	10	28	25	91	76
	0	2	15	14	61	50
	4	6	6	6	75	64

nd, not done.
( ) number of parasites collected from the peritoneum.

($P < 0.05$) than those of the ITTn sheep. Three of the four ITTe sheep had values below 50 units/l until nine weeks after infection. The other ITTe sheep had higher values from the fifth week and died of fasciolosis one week after the experiment finished. That sheep developed a low pcv at 10 weeks and was the only ITT sheep, naive or exposed, to die of fasciolosis in the total of 84 sheep for which resistance has been assessed in our studies (Wiedosari and Copeman, 1990; Roberts et al., 1997a,b and Roberts, Estuningsih, Widjayanti, Wiedosari, Spithill, 1994, unpublished results).

The mean plasma γGT values of the same groups of sheep were consistently lower than 80 units/l, and there were no significant differences between the groups.

Table 2
The effect of dexamethasone on the resistance of ITT (exposed) sheep challenged with 200 metacercariae of *F. gigantica*. Resistance was suppressed by the dexamethasone

	Commencement of dexamethasone administration (days after infection)				No dexamethasone	
	−2	3	6	12		
	Number of parasites				ITT sheep	JFT sheep
Mean	27.5 a	18.0 ab	12.8 a	14.8 a	2.3 b	19.3 a
Sd	10.3	21.2	7.0	9.1	1.9	13.2

Means with different letters are significantly different ($P < 0.05$).

3.2. Dynamics of infection, Experiment 2

Parasite counts were consistent with lesion counts, but a lower proportion of early parasites was obtained because of difficulties in collecting very small parasites, Table 1. Significantly fewer lesions were observed on the livers from ITTe sheep than from either of the other groups ($P < 0.05$), and from three weeks there were fewer observed from the ITTn sheep than from the Merinos ($P < 0.01$). Most of the lesions that would be present 42 days after infection in ITTn sheep, and in Merinos, were present at 14 days, whereas it took 28 to 42 days for that to occur in ITTe sheep (Table 1). From two weeks after infection, there were significantly fewer lesions observed on the livers from ITTe sheep than from either of the other groups ($P < 0.05$), and from three weeks there were fewer observed from the ITTn sheep than from the Merinos ($P < 0.01$). The only parasites collected from the peritoneum were two from a Merino sheep 10 days after infection.

The parasites from the ITTn sheep were significantly smaller than those from the Merino sheep 14 ($P < 0.01$) and 21 to 42 ($P < 0.001$) days after infection (Table 3). There was no significant difference between the sizes of the parasites from the ITTe and ITTn sheep 42 days after infection, when the parasites from the ITTe sheep were also smaller ($P < 0.001$) than those from the Merinos.

The lesions were convoluted tracts, 1–2 mm diameter, usually just below the capsule. They were cream coloured when they first appeared 14 days after infection, but developed a green hue from four weeks. There were no fibrin tags or other macroscopic changes to the liver capsule, and surface lesions were evenly distributed between parietal and visceral surfaces, and dorsal and ventral lobes.

3.3. Effect of dexamethasone on infections in ITTe sheep, Experiment 3

Dexamethasone substantially reduced the resistance of ITTe sheep when administration commenced up to 12 days after infection (Table 2) there being a significant difference between the group of untreated ITTe sheep and the group given dexamethasone prior to infection ($P < 0.01$), and also two of the three groups of ITTe given

Table 3
Lengths (mm) of *F. gigantica* parasites collected at intervals after infection of ITT (exposed), ITT (naive) and Merino (naive) sheep with 500 metacercariae

Age (days)	Length (mean ± sd)		
	ITT (exposed)	ITT (naive)	Merino (naive)
10	Nil	0.25 (1)	0.28 ± 0.70 (13) 0.30, 0.35 from peritoneum
14	0.30, 0.35 (2)[a]	0.48 ± 0.14 (5)a	0.68 ± 0.13 (40)b
21	1.30 (1)	1.85 ± 0.63 (19)a	3.05 ± 1.39 (155)b
28	1.85, 2.10 (2)	2.41 ± 0.61 (9)a	3.54 ± 0.78 (40)b
42	3.38 ± 0.91 (9)a	3.10 ± 1.26 (30)a	5.18 ± 1.36 (206)b

[a] Number of intact parasites measured.
Values in rows with different letters are significanly different ($P < 0.01$).
Parasites less than 21 days old were fixed and older parasites were measured fresh.

dexamethasone after infection ($P < 0.05$). The number of parasites in the control group of naive JFT sheep was not significantly different from that in any of the dexamethasone-treated ITTe groups.

4. Discussion

Damage to liver parenchymal cells caused by migrating and feeding juvenile *Fasciola* parasites (Sinclair, 1973), elevates plasma GLDH levels (Anderson et al., 1977), so they were used to assess the timing and amount of damage caused by juvenile parasites in the livers. The values measured (Fig. 1), indicated that there was little damage in the livers of ITTe sheep four weeks after infection, whereas there was early and substantial damage to liver parenchymal cells in ITTn and Merino sheep. Thus, it appeared that many juvenile parasites were not reaching the livers of ITTe sheep. The consistently low values for plasma γGT indicate that there was no appreciable damage to bile ducts during the period studied (Anderson et al., 1977).

Most early parasite tracts in the livers of sheep were visible through the capsule in infections with *F. hepatica* (Dow et al., 1968) and *F. gigantica* (Wiedosari et al., 1991), so surface lesions were counted as indexes of the sizes of parasite populations from two weeks, when tracts first appeared in sheep (Dow et al., 1968; Rushton, 1977; Table 1). Counts of surface tracts and collections of parasites (Table 1), and growth rates of parasites (Table 3), all confirmed that there was inhibition of the migration and development of juvenile parasites in ITTe sheep within two weeks of infection, indicating that resistance was acquired. At that time parasite numbers and growth rates in ITTn sheep were intermediate between those in ITTe and Merino sheep (Tables 1 and 3), presumably because the ITTn sheep were acquiring resistance. Thus, the study of the dynamics of early infections further indicated that an acquired resistance mechanism had prevented most parasites from migrating to the livers of ITTe sheep. A protective immune response could have developed by 14 days after infection because by then circulating antigens of *F. hepatica* had been detected in sheep (Rodriguez-Pérez and Hillyer, 1995), and humoral (Tiggele and Over, 1976; Oldham, 1983), and cellular responses (Oldham and Williams, 1985) had developed against that parasite.

The proportions of the infecting dose recovered from all groups of sheep 21 to 28 days after infection (Table 1) were similar to recoveries of mature parasites from comparable groups (Roberts et al., 1997a), so most killing of parasites had probably ceased by then. Thus, parasites up to that age had stimulated the immune system with the critical antigen, and were vulnerable to the effects of the response. Consequently, early juvenile parasites should be studied with a view to identifying the critical antigen for vaccine trials. Somatic and excretory–secretory antigens from 16-day-old *F. hepatica* protected mice (Lang and Hall, 1977).

The resistance of the ITTe sheep was suppressed by dexamethasone (Table 2), providing further evidence that it was acquired. Until at least 12 days after infection of ITTe sheep, approximately the same number of parasites, which eventually persisted in the control JFT breed, were still alive, because they were 'rescued' by administration of dexamethasone to the hosts (Table 2). Only about 12% of them would have survived in

untreated ITTe sheep (Table 2). The migration of *Fasciola* in ruminants is generally accepted as through the wall of the jejunum, across the surface of the peritoneum, and into the liver through the capsule (Doy and Hughes, 1984). Sogoyan (1955) considered the route from the jejunum via the portal vein to be more important. In either case, as the inhibited parasites in ITTe sheep were not detected on the peritoneal surface, or in the liver (Table 1), they were probably in the wall of the jejunum. Similarly, in rats, fewer juvenile *F. hepatica* penetrated the serosa of the intestine after secondary infection (Hayes and Mitrovic, 1977). Acquired resistance against *F. hepatica* in rats was associated with anaphylaxis and infiltration of the intestine with eosinophils (Doy et al., 1981). Moreover, eosinophils adhered to newly excysted juveniles, and damaged their tegument, when they were injected intraperitoneally into sensitised rats (Davies and Goose, 1981). Eosinophilia was also an early feature of infections of ITTn sheep with *F. gigantica* (Wiedosari et al., 1991; Roberts et al., 1997a), so the role of eosinophils in the ITT resistance system should be studied.

The basis for the high resistance of challenged ITTe sheep is probably either a protective immune reaction unique to the ITT:*F. gigantica* relationship, or a difference in the activity of a component of the effector arm of an immune response, such as eosinophil activity. As ITT sheep acquired resistance against *F. gigantica*, but not against *F. hepatica* (Roberts et al., 1997a), it is unlikely that the critical component of resistance lay in the effector arm of the immune response. Further, as the major resistance mechanism against *F. gigantica* was acquired by ITT sheep, but not by Merinos, it is possible that ITT sheep have an exceptional capacity to respond to a critical antigen that is peculiar to *F. gigantica*. A complementary hypothesis would be an immune reaction peculiar to ITT sheep, such as those against *F. hepatica*, which inhibited an immunological suppressor (Cervi et al., 1996), and an antibody inactivator (Carmona et al., 1993).

Acknowledgements

The *Fasciola* project at Balitvet was financed by the Australian Centre for International Agricultural Research. Support for the project by the Coordinator of the Department of Parasitology, Dr. Sutijono Partoutomo, is gratefully acknowledged. The careful technical assistance of Yayan, Suhayanto and Soedrajat is appreciated.

References

Altaif, K.I., Dargie, J.D., 1978. Genetic resistance to helminths: the influence of breed and haemoglobin type on the response to reinfection with *Haemonchus contortus*. Parasitology 77, 177–187.

Anderson, P.H., Berret, S., Brush, P.J., Hebert, C.N., Parfitt, J.W., Patterson, D.S.P., 1977. Biochemical indicators of liver injury in calves with experimental fasciolosis. Vet. Rec. 100, 43–44.

Boray, J.C., 1967. Studies on experimental infections with *Fasciola hepatica*, with particular reference to acute fascioliasis in sheep. Ann. Trop. Med. Parasitol. 61, 439–450.

Carmona, C., Dowd, A.J., Smith, A.M., Dalton, J.P., 1993. Cathepsin L proteinase secreted by *Fasciola hepatica* in vitro prevents antibody-mediated eosinophil attachment to newly excysted juveniles. Mol. Biochem. Parasitol. 62, 9–18.

Cervi, L., Rubinstein, H., Masih, D.T., 1996. Involvement of excretion–secretion products from *Fasciola hepatica* inducing suppression of the cellular immune responses. Vet. Parasitol. 61, 97–111.

Davies, C., Goose, J., 1981. Killing of newly excysted juveniles of *Fasciola hepatica* in sensitized rats. Parasitol. Immunol. 3, 81–96.

Dow, C., Ross, J.G., Todd, J.R., 1968. The histopathology of *Fasciola hepatica* infections in sheep. Parasitology 58, 129–135.

Doy, T.G., Hughes, D.L., 1984. Early migration of immature *Fasciola hepatica* and asssociated liver pathology in cattle. Res. Vet. Sci. 37, 219–222.

Doy, T.G., Hughes, D.L., Harness, E., 1981. Hypersensitivity in rats infected with *Fasciola hepatica*: possible role in protection against a challenge infection. Res. Vet. Sci. 30, 360–363.

Farrell, C.J., Shen, D.T., Wescott, R.B., Lang, B.Z., 1981. An enzyme-linked immunosorbent assay for diagnosis of *Fasciola hepatica* infection in cattle. Am. J. Vet. Res. 42, 237–240.

Hayes, T.J., Mitrovic, M., 1977. The early expression of protective immunity to *Fasciola hepatica* in rats. J. Parasitol. 63, 584–587.

Lang, B.Z., Hall, R.F., 1977. Host–parasite relationships of *Fasciola hepatica* in the white mouse: VII. Successful vaccination with culture incubate antigens and antigens from sonic disruption of immature worms. J. Parasitol. 63, 1046–1049.

Oldham, G., 1983. Antibodies to *Fasciola hepatica* antigens during experimental infections in cattle measured by ELISA. Vet. Parasitol. 13, 151–158.

Oldham, G., Williams, L., 1985. Cell mediated immunity to liver fluke antigens during experimental *Fasciola hepatica* infection in cattle. Parasitol. Immunol. 7, 503–516.

Roberts, J.A., 1968. Acquisition by the host of resistance to the cattle tick *Boophilus microplus* (Canestrini). J. Parasitol. 54, 657–662.

Roberts, J.A., Widjayanti, S., Estuningsih, E., 1996. Acquired resistance of Merino sheep against *Fasciola gigantica*. Parasitol. Res. 82, 743–746.

Roberts, J.A., Estuningsih, E., Widjayanti, S., Wiedosari, E., Partoutomo, S., Spithill, T.W., 1997a. Resistance of Indonesian Thin Tail sheep against *Fasciola gigantica* and *F. hepatica*. Vet. Parasitol. 68, 69–78.

Roberts, J.A., Widjayanti, S., Estuningsih, E., Hetzel, D.J., 1997b. Evidence for a major gene determining the resistance of Indonesian thin tail sheep against *Fasciola gigantica*. Vet. Parasitol. 68, 309–314.

Rodriguez-Pérez, J., Hillyer, G.V., 1995. Detection of excretory–secretory circulating antigens in sheep infected with *Fasciola hepatica* and with *Schistosoma mansoni* and *F. hepatica*. Vet. Parasitol. 56, 57–66.

Rushton, B., 1977. Ovine fasciolosis following reinfection. Res. Vet. Sci. 22, 133–134.

Sinclair, K.B., 1973. The resistance of sheep to *Fasciola hepatica*: studies on the development and pathogenicity of challenge infections. Br. Vet. J. 129, 236–250.

Sogoyan, I.S., 1955. Pathology of experimental fasciolosis in sheep caused by *Fasciola gigantica*. Trudy Armyanskogo Nauchno-issledovatel'-skogo Veterinarnogo Instituta 8, 155–165, [Russian].

Tiggele, L.J. van, Over, H.J., 1976. Serological diagnosis of fascioliasis. Vet. Parasitol. 1, 239–248.

Wiedosari, E., Copeman, D.B., 1990. High resistance to experimental infection with *Fasciola gigantica* in Javanese thin-tailed sheep. Vet. Parasitol. 37, 101–111.

Wiedosari, E., Graydon, R., Copeman, D.B., 1991. Comparative pathological study of hepatic changes induced by *Fasciola gigantica* and *Gigantocotyle explanatum* in Javanese thin-tailed sheep. J. Comp. Pathol. 105, 147–155.