Parasitol Res (2003) 91: 27–33
DOI 10.1007/s00436-003-0828-3

ORIGINAL PAPER

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Parasitological and morphological findings in porcine isosporosis after treatment with symmetrical triazintriones

Received: 11 December 2002 / Accepted: 11 December 2002 / Published online: 11 July 2003

Abstract Neonatal porcine isosporosis is known to cause serious economic losses in piglet farms by causing severe enteritis with dehydration, weight loss and reduced development in the affected animals, predominantly during the first weeks of life. In the present study, piglets experimentally infected with *Isospora suis* were treated with Bay Vi 9143, a symmetrical triazintrione, at different days post-infection. As shown by clinical and pathological examinations, Bay Vi 9143 is effective against the asexual and sexual stages of *I. suis* at all selected treatment times. However, the therapeutic use at an early stage of asexual multiplication is most effective before the onset of clinical symptoms.

Introduction

Isosporosis in piglets is caused by infection with the coccidium *Isospora suis*, a primary pathogen that was first described 1934 by Biester and Murray. Piglets are exposed to sources of infection from birth, becoming infected by ingesting sporulated oocysts. Since the prepatent period of *I. suis* is short at about 5 days, conditions occur which allow an infection to develop rapidly in the first 2 weeks of life. The disease usually manifests clinically from the age of about 8–10 days. Its course is characterised by diarrhoea, dehydration, in some cases secondary infections, and occasionally death. Affected animals fail to gain body mass and litters develop inconsistently until they are weaned (Matuschka and Heydorn 1980; Matuschka 1982; Mundt and Koudela 2001).

The aim of the present investigation was to determine the optimal time of treatment for isosporosis with a member of the class of symmetrical triazintriones, Bay Vi 9143.

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Materials and methods

Experimental design

The investigations were carried out using piglets artificially infected with *I. suis* in a blind study.

Sows were housed conventionally 3 weeks before the calculated farrowing date. The piglets received prophylactic iron supplement therapy post-partum (at the age of 2 days, s.c.). Piglets from four litters were randomly distributed into four groups (eight piglets/group). At the age of 3 days, all of the piglets were orally infected with 10^4 sporulated oocysts of a pathogenic field isolate of *I. suis* obtained from a piglet-rearing farm in northern Germany.

Each experimental group of eight animals was given 20 mg/kg body weight Bay Vi 9143 on day 2 post-infection (p.i.) (treatment group T1), on day 3 p.i. (treatment group T2) and on day 4 p.i. (treatment group T3). The remaining group of eight animals served as a positive control and were not treated (control group C). Instead of the test substance, they received tap water. Bay Vi 9143 was administered orally as a 5% suspension.

Additionally, the piglets of all groups were divided into subgroups, each consisting of four animals, assigned for necropsy on day 8 or 10, respectively. Thus, each subgroup contained four piglets (Table 1).

Clinical and parasitological examination

The piglets' general state of health was assessed and the following clinical and parasitological parameters were determined: faecal consistency was assessed and oocyst excretion (oocytes/g) was determined from individual rectal faecal samples taken once daily on the day of infection (at the age of 3 days) and on days 4–7 p.i.

Table 1 Experimental design

Experimental group	Dose (mg/kg)	Treatment day p.i.	Age (days) at necropsy on day 8	Age (days) at necropsy on day 10
C (control)	–	2	5	7
T1 Bay Vi 9143	20	2	5	7
T2 Bay Vi 9143	20	3	6	8
T3 Bay Vi 9143	20	4	7	9

The consistency of the faeces was assessed using the following scale: (1) firm and formed, (2) pasty, (3) semi-liquid and (4) liquid.

Oocyst determination was carried out by flotation in saturated sodium chloride solution with glucose (Henriksen and Christensen 1992) and oocytes were counted in a modified McMaster chamber.

Necropsy and histological technique

For necropsy, the animals were killed by exsanguination after anaesthesia by captive bolt for small animals. The intestine was exenterated beginning with the ileum at the entrance into the caecum. Five pieces of approximately 20 cm length were taken, rinsed with phosphate buffered saline (PBS) and subsequently fixed intraluminally with 10% neutral buffered formalin. The proximal and distal ends of the samples were closed with plastic strips, and the samples postfixed by immersion in 10% neutral buffered formalin. Transverse cuts of the proximal, middle and distal parts of these gut samples, as well as a longitudinal cut of the middle part, were prepared. All samples were embedded in paraffin, cut at a thickness of approximately 5 μm and stained with haematoxylin and eosin (H&E).

All slides from the five intestinal locations collected were examined histopathologically, beginning with sample 1, which was the piece of gut taken next to the entrance of the ileum into the caecum, and ending with sample 5, the piece taken furthest from the caecal entrance. The findings from the transverse sections as well as from the longitudinal section were summarised and recorded under a severity grade representing the most severe pathological alteration.

The criteria for the histopathological evaluation were: (1) atrophy and/or fibrinoid necrosis of the villi, (2) inflammatory reaction characterised by an increased number of single cell necroses in the villous tips and granulocytic infiltration of the villi, and (3) the presence of parasite stages with different development stages (schizonts, macro- and microgamonts, and oocysts (Figs. 1, 2).

Electron microscopy

Additional samples of the small intestine were taken for scanning electron microscopy (between sample 1 and 2 taken for histopathology). The samples were clamped on cork plates, flushed with tap water and fixed with 2% glutaraldehyde in phosphate buffer. They were secondarily fixed with 1% osmium tetroxide solution for 2 h. Thereafter, samples from selected animals with characteristic histopathological findings were dehydrated, mounted on specimen holders, sputtered with gold and examined using a Philips scanning electron microscope WL 30.

Data processing

For unbiased histopathological examination, the piglets were divided into two subgroups according to the different times of necropsy. Only when the histopathological evaluation was finished, the study was deblinded and the animals assigned to their treatment groups.

Results

Clinical and parasitological findings

The field isolate used proved to be pathogenic. Infection of the untreated control animals led to the characteristic clinical picture of isosporosis. At the age of 7 days

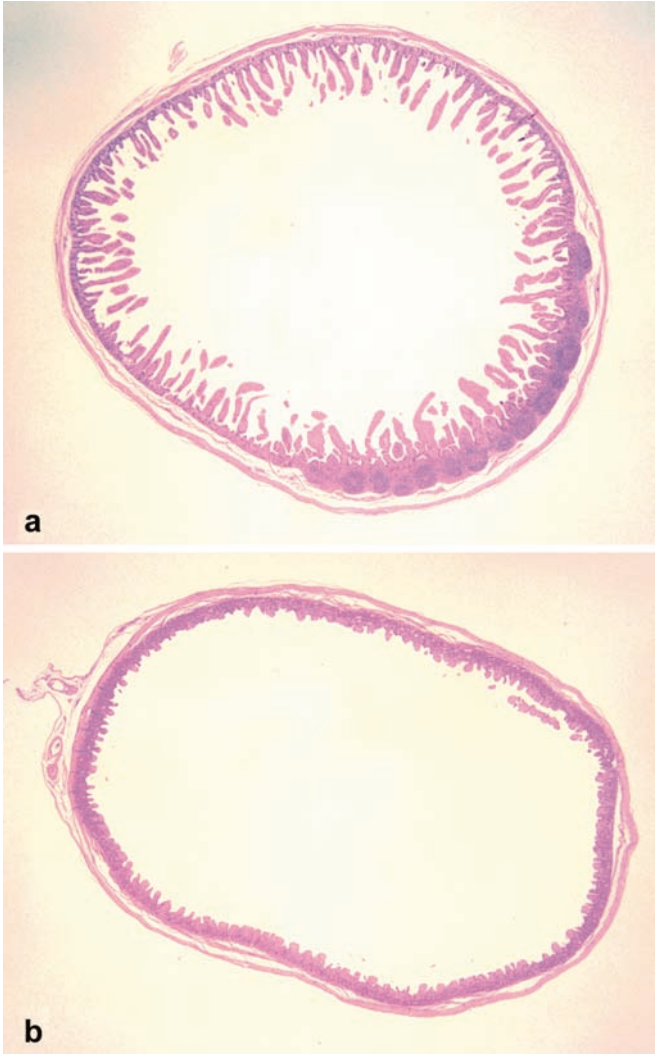

Fig. 1a, b Day 5 of necropsy, H&E stain. **a** day 5 p.i., unaffected small intestine with normal villous length, 12×. **b**: Day 5p.i., severe villous atrophy, 12×

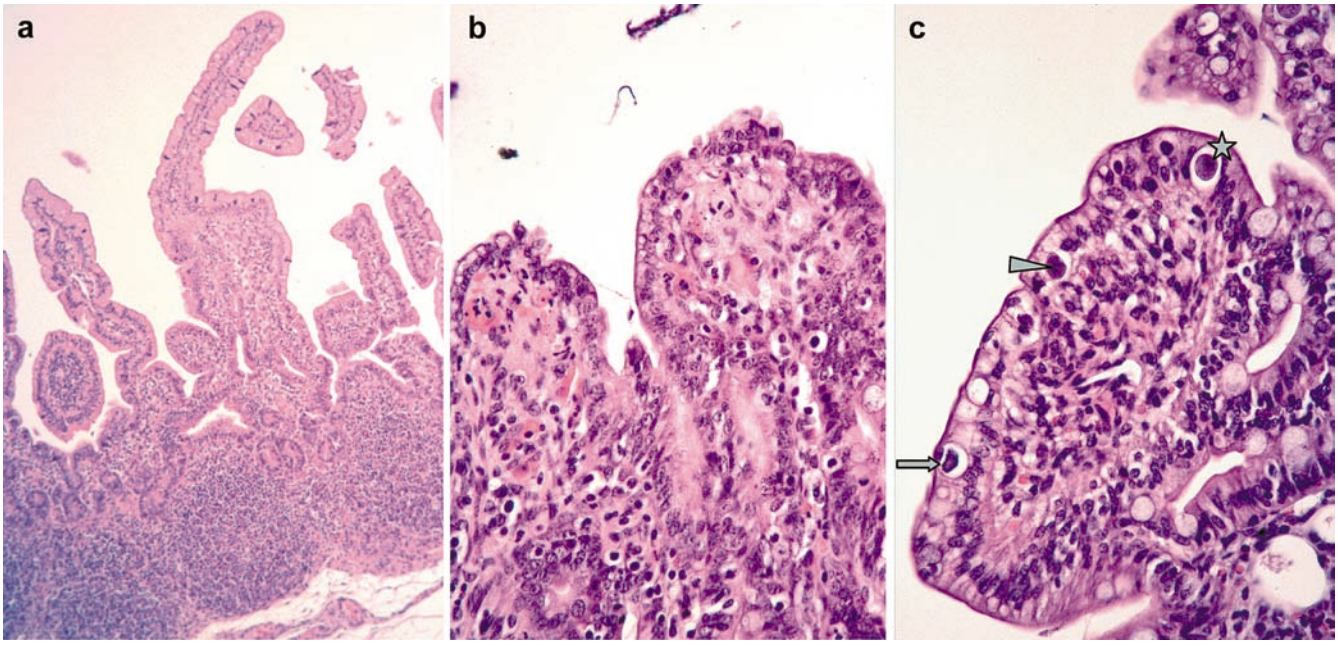

Fig. 2a–c Day 8 of necropsy, H&E stain. **a** Day 5 p.i., unaffected villi of the small intestine with underlying lymphoid tissue, 75×. **b** Infected untreated control, atrophic villi of the small intestine with fibrinoid necrosis of the tip, 300×. **c** Infected, untreated control, villous tip with different intraepithelial parasite stages, microgamont (*arrow*), macrogamont (*arrowhead*), oocyst (*star*), 300×

(4 days p.i.) the diarrhoea typically associated with isosporosis was observed, with yellowish, semi-liquid to liquid faeces containing no blood. *I. suis* oocysts were detectable in the faeces from the age of 8 days (5 days p.i.). After early treatment (48 h p.i.) neither the clinical symptoms (diarrhoea) nor oocyst excretion were seen (Figs. 3, 4). Treatments given later, by their very nature, had no influence on the clinical changes caused by the infection, although oocyst excretion was reduced considerably.

Gross findings

At necropsy on day 8, all animals from the control group revealed a change of intestinal contents. Two of these piglets had yellowish, watery ingesta, one piglet showed a reddish discoloration of the mucosa of the small intestine, and in one the gut was filled with gas. None of the animals from group T1 showed any macroscopic findings in the gut. In one animal from this group, slightly enlarged mesenteric lymph nodes were recorded. One piglet from group T2 had gas-filled intestines, another animal from this group had watery yellowish ingesta as did one piglet from group T3.

At necropsy on day 10, the gut of all animals (control and treated) appeared normal. Slightly enlarged mesenteric lymph nodes were seen in only one animal from the treatment groups.

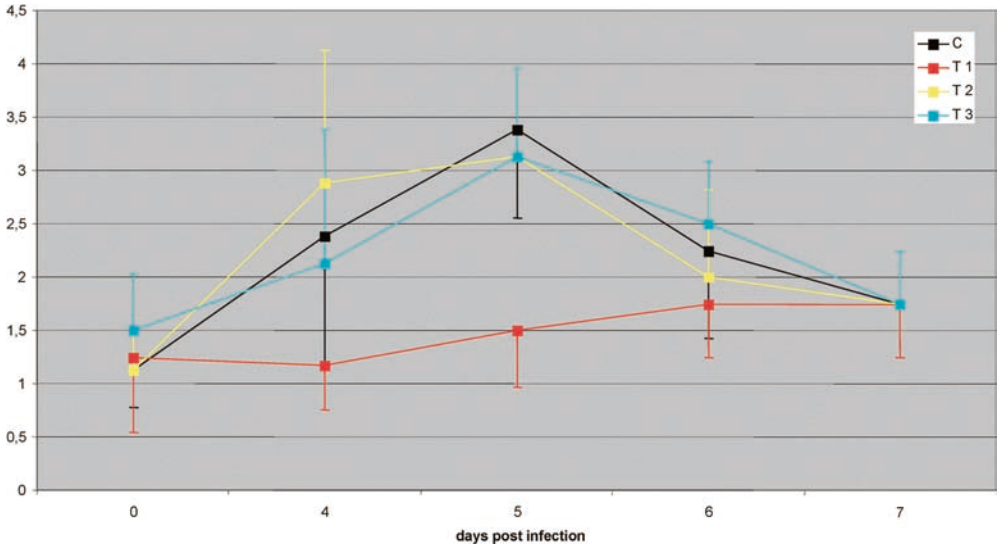

Fig. 3 Faecal score of piglets (average per group)

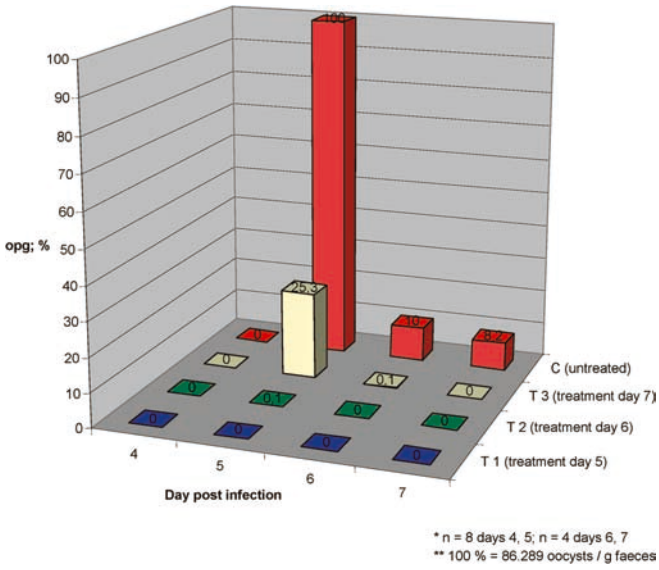

Fig. 4 Average faecal oocyst excretion in % oocytes/g (*opg*)

Histopathological examination

Day 8

All animals from the control group showed atrophy of the villi with fibrinoid necrosis and signs of inflammatory reactions (Figs. 1b, 2b). Different parasite stages were also seen in all of these animals (Fig. 2c). Frequently, the findings were more pronounced in the distal parts of the small intestine (samples 1–3, Table 2).

Only one piglet from the T1 group showed minimal atrophy and fibrinoid necrosis of the small intestinal villi with inflammatory reactions. Another animal from this group showed a minimal occurrence of parasites, however, only in one single location (sample 5, Table 2).

All animals from the T2 group showed fibrinoid necrosis of the villous tips together with shortened villi and different parasite stages in multiple locations (Table 2).

Similar findings were seen in the animals from the T3 group. All piglets revealed fibrinoid necrosis and parasite stages in different locations. Three piglets had shortened intestinal villi.

Day 10

In all animals from the untreated control group, atrophy of the villi with or without fibrinoid necrosis was observed. In one of the animals, this finding was accompanied by an inflammatory reaction. Two piglets contained parasite stages (Table 3). Only one animal from group T1 revealed atrophy of the villous tips with fibrinoid necrosis. Parasite stages were obvious in the same animal as well as in one other piglet from this group. Signs of inflammatory reaction occurred only to a minimal degree in one animal and in one location (sample 5, Table 3). Three piglets from group T2 showed minimal fibrinoid necrosis of the villous tips. In two of these animals the villi were also shortened. No parasite stages were observed in any of the four animals in this group (Table 3). All piglets from the T3 group had shortened villi and three also had fibrinoid necrosis of the tips. In one animal parasite stages were present in a single location (sample 1, Table 3).

Histopathological evaluation revealed that there were no differences in the gut-associated lymphoid tissue between the animals of the different groups.

Electron microscopy

At day 8, animals from the untreated control group showed a damaged epithelium of the villous tips

Table 2 Histopathological findings in control and treated animals, necropsy on day 8, the mean score of all animals per group is given. *C* Control group, *T* treatment group. Severity of histopathological findings: +/– minimal, + slight, ++ moderate, +++ marked

Sample no.	Experimental group	Atrophy/necrosis	Inflammatory reaction	Parasite stages
1	C	++	+	++
	T1	+/–	+/–	–
	T2	+	+	+
	T3	+	+	++
2	C	+++	++	++
	T1	–	+/–	+/–
	T2	+++	+	++
	T3	++	+	++
3	C	+++	++	++
	T1	+/–	+/–	–
	T2	+++	+	+/–
	T3	++	+	++
4	C	+++	++	++
	T1	+/–	+/–	–
	T2	+++	++	+/–
	T3	++	+	+
5	C	+++	++	+
	T1	–	+/–	+/–
	T2	+++	++	+/–
	T3	++	++	+

Table 3 Histopathological findings in control and treated animals, necropsy on day 10, the mean score of all animals per group is given. *C* Control group, *T* treatment group. Severity of histopathological findings: +/– minimal, + slight, + + moderate, + + + marked

Sample no.	Experimental group	Atrophy/necrosis	Inflammatory reaction	Parasite stages
1	C	+	+/–	–
	T1	–	–	–
	T2	+/–	–	–
	T3	+	+/–	+/–
2	C	+	+/–	+/–
	T1	–	–	–
	T2	+	–	–
	T3	+	–	–
3	C	+	+/–	–
	T1	–	–	–
	T2	+	+/–	–
	T3	+	+/–	–
4	C	+ +	+	+/–
	T1	–	–	–
	T2	+ +	+/–	–
	T3	+ +	+/–	–
5	C	+ +	+/–	+/–
	T1	+/–	+/–	+/–
	T2	+	+/–	–
	T3	+ +	–	–

(Fig. 5b). The affected cells were rounded, detached from the basal membrane or desquamated. In some cases, ruptured epithelial cells—due to the release of parasite stages—were observed (Fig. 6a–d). At day 10, the epithelial layer of the villi of infected animals was regenerated. However, the villi were still shortened and had a plump appearance (Fig. 7).

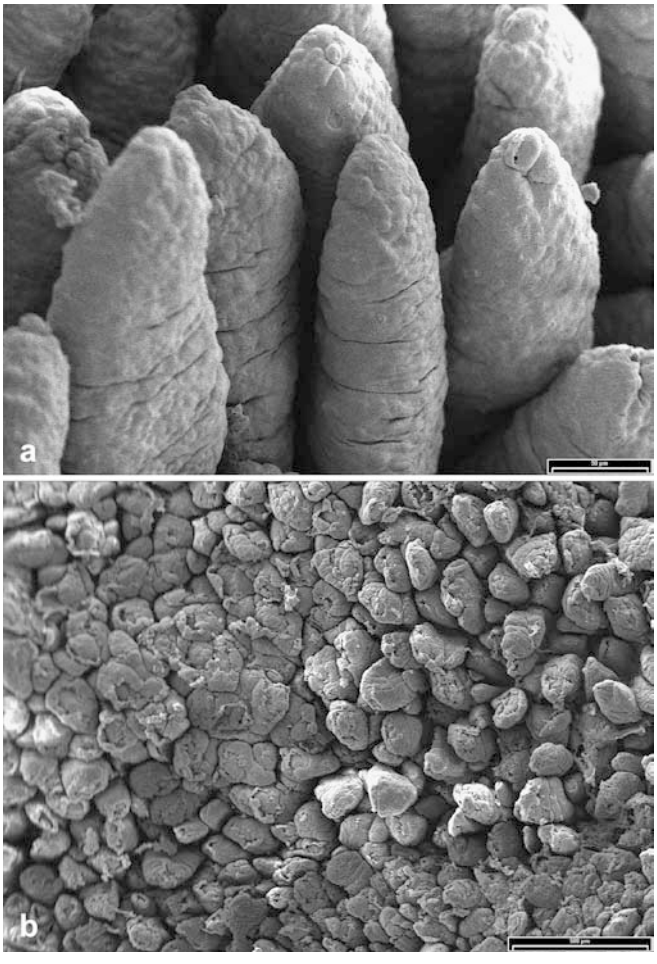

Fig. 5a, b Day 8 of necropsy. SEM corresponding to Fig. 1a, b. **a** Unaffected villi, **b** severe villous atrophy

Discussion

Neonatal isosporosis is a widespread disease in piglets which is known to cause considerable economic loss through a delay in neonatal development and reduced body weight gain (Matuschka and Heydorn 1980). Mortality, however, is generally low. The gross pathological changes are the morphological and functional basis for the symptoms and the economic significance of piglet coccidiosis. During the acute phase of tissue damage, scouring is the clinical lead symptom. Due to impaired digestive and absorptive activity in the small intestine caused by the severe atrophy of the intestinal villi (Matuschka 1982), affected animals develop more slowly and show a delay in their body weight development. Digestive function is evidently still reduced even when re-epithelialisation is complete. However, as the protective function of the intestinal epithelium is severely impaired, secondary infections can considerably complicate the clinical picture under field conditions (Driesen et al. 1995).

In this study, the clinical features of the infectious process caused by the selected strain of *I. suis* were equal to natural infections encountered in farrowing units. The field strain, isolated in northern Germany, was found to be pathogenic (10^4 sporulated oocysts per animal at 3 days of age). Infected, untreated control animals developed the characteristic clinical and pathological signs of isosporosis, i.e. diarrhoea with yellowish, watery ingesta, discoloration of the mucosa and/or a gas filled gut. Histopathologically, the typical findings of villous atrophy as a result of fibrinoid necrosis and

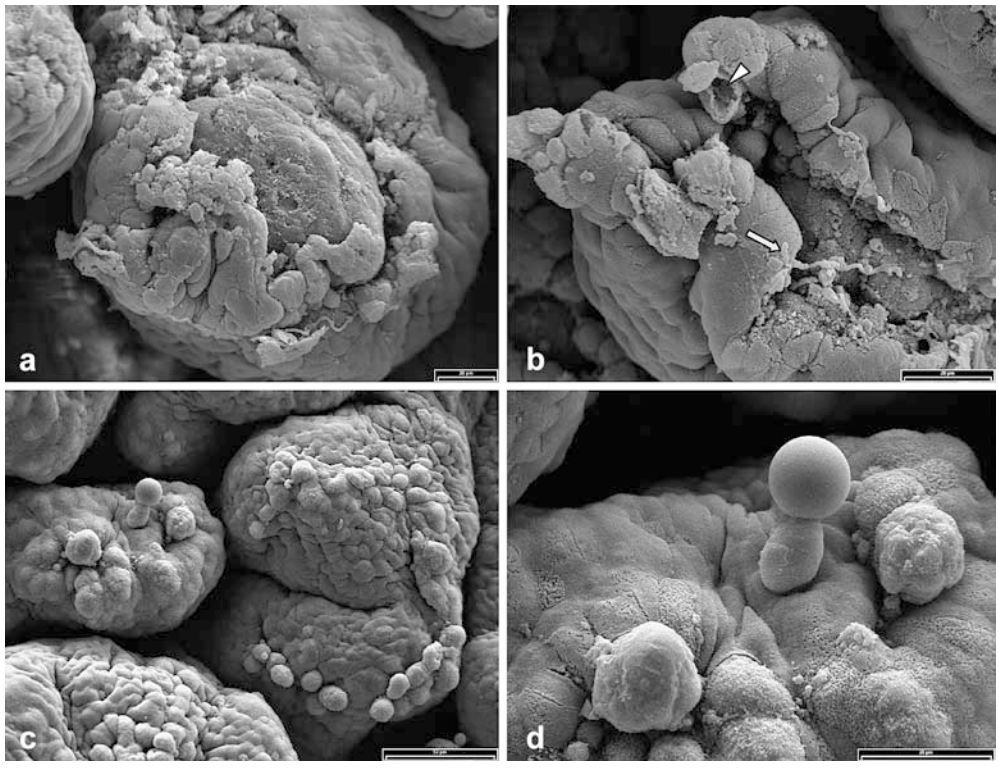

Fig. 6a–d Day 5 p.i. SEM. **a** Epithelial desquamation with underlying unprotected villous stroma, **b** detached epithelium with empty epithelial cell (parasitic stage released, *arrowhead*) and fibrin (*arrow*), **c** desquamation of enterocytes, villous tip, **d** released oocyst

sloughing of epithelial cells, inflammatory reaction and the presence of different parasite stages within the intestinal epithelium could be seen as described previously (Stuart et al. 1980; Chae et al. 1998). The study also confirmed previous data on the mode of action of symmetrical triazintriones against protozoa (Mehlhorn et al. 1984; Harder and Haberkorn 1989; Varga 1989; Hackstein et al. 1995). Clinical, parasitological as well as light and electron microscopic findings from studies of *Eimeria* spp. in chickens have shown that all intracellular developmental stages of the parasites are damaged by the compound, i.e. all stages of the asexual (merogony) and sexual multiplication (gametogony). Accordingly, in this study with *I. suis* a therapeutic concentration of Bay Vi 9143 (20 mg/kg body weight) proved fully effective in all cases and at all treatment times (days 2–4 p. i.). After the early treatment at 2 days p. i., no clinical signs or oocyst excretion occurred. Histopathologically, parasitic stages, fibrinoid necroses of the villous tips or villous atrophy were seen in only one or very few locations and to a low extent. Thus, at this time tissue damage was restricted to a minimal degree by the early treatment with the compound. A later treatment on day 3 p. i. also led to a disruption of parasitic development (no oocyst excretion), but tissue damage had already been caused prior to treatment with a much higher severity. The lesions were manifest clinically (altered faecal consistency, scouring) and pathologically (fibrinoid necroses and atrophy of the villi, inflammatory reaction and parasite stages in all animals at different locations). A similar clinical and pathological picture, with correspondingly more advanced signs,

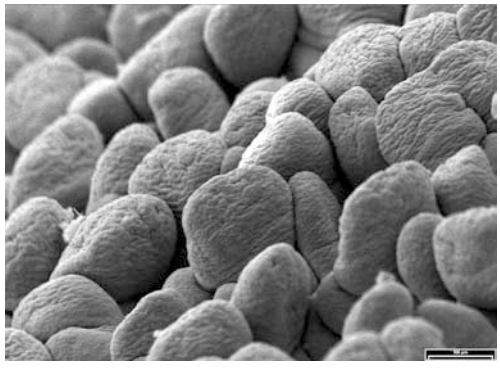

Fig. 7 Day 7 p.i. SEM. Re-epithelialisation of villi. The villi are still considerably shortened when compared to unaffected examples (Fig. 44)

emerged when treatment was even later (day 4 p. i.). At this time, the first parasite stages had undergone sexual multiplication and some animals were already excreting oocysts. Yet, even this late treatment suppressed oocyst excretion to a large extent as is demonstrated by the comparison with the untreated infected control animals. Comparing the results of the pathological examination after necropsy on days 8 and 10, respectively, the differences in untreated and treated animals were not as clear in animals necropsied on day 10 when compared to animals killed after 8 days.

We conclude that Bay Vi 9143, a member of the symmetrical triazintriones, is effective against the asexual and sexual stages of *I. suis*. Thus, the activity profile against *I. suis* in suckling pigs is comparable to that

against *Eimeria* spp. in chickens. This has important implications for the recommendations on the practical use of this class of compounds. They stop parasitic development irrespective of the progression of the disease, thereby minimising further losses. The therapeutic use at an early stage of the infection process before the onset of clinical symptoms was found to be most valuable with the development of the parasite being suppressed before major tissue damage occurred. For this reason, treatment of piglet coccidiosis under field conditions is recommended during the first few days of life (Madsen et al. 1994; Martineau et al. 1994; Mundt 2000).

Acknowledgements The authors wish to thank Petra Hartmann, Alfred Leufgen and Meike Petersen for excellent technical assistance. The animal experiments mentioned in this paper comply with current German laws.

References

Biester HE, Murray C (1934) Studies in infectious enteritis of swine. VIII. *Isospora suis* n. sp. in swine. J Am Vet Med Assoc 85:207–219

Chae C, Kwon D, Kim O, Min K, Cheon DS, Choi C, Kim B, Suh J (1998) Diarrhoea in nursing piglets associated with coccidiosis: prevalence, microscopic lesions and coexisting microorganisms.Vet Rec 143:417–420

Driesen SJ, Fahy VA, Carland PG (1995) The use of toltrazuril for the prevention of coccidiosis in piglets before weaning. Aust Vet J 72:139–141

Hackstein JHP, Mackenstedt U, Mehlhorn H, Meijerink H, Schubert H, Leunissen JAM (1995) Parasitic apicomplexans harbour a chlorophyll a-D1 complex, the potential target for therapeutic triazines. Parasitol Res 81:207–216

Harder A, Haberkorn A (1989) Possible mode of action of toltrazuril: studies on two *Emeria* species and mammalian *Ascaris suum* enzymes. Parasitol Res 76:8–12

Henriksen SA, Christensen JPB (1992) Demonstration of *Isospora suis* oocysts in faecal samples. Vet Rec 131:443–444

Madsen P, Henriksen SVAA, Larsen K (1994) Prophylactic treatment of *Isospora suis* coccidiosis in piglets with toltrazuril in a special oral suspension. Proceedings of the International Pig Veterinary Society, 26–30 June, Bangkok, Thailand, p 256

Martineau GP, Menard J, Carabin H, Villeneuve A, Dumas G (1994) Strategic control of porcine coccidiosis with Toltrazuril. Proceedings of the International Pig Veterinary Society, 26–30 June, Bangkok, Thailand, p 243

Matuschka FR (1982) Ultrastructural evidence of endodyogeny by *Isospora suis* in pigs. Z Parasitenkd 67:27–30

Matuschka FR, Heydorn AO (1980) Die Entwicklung von *Isospora suis* Biester und Murray 1934 (Sporozoa: Coccidia: Eimeriidae) im Schwein. Zool Beitr NF 26:405–476

Mehlhorn H, Ortmann-Falkenstein G, Haberkorn A (1984) The effects of sym. Triazinones on developmental stages of *Eimeria tenella*, *E. maxima* and *E. acervulina*: a light and electron microscopical study. Z Parasitenkd 70:173–182

Mundt HC (2000) Baycox 5% an anticoccidial for the treatment of *Isospora suis* coccidiosis in piglets.Proceedings of the 16th International Pig Veterinary Society Congress, Melbourne, Australia, p 80

Mundt HC, Koudela B (2001) Don't forget coccidiosis. Pig Prog 17:2–4

Stuart BP, Lindsay DS, Ernst JV, Gosser HS (1980) *Isospora suis* enteritis in piglets. Vet Pathol 17:84–93

Varga I (1989) The timing of the treatment of chickens with toltrazuril against coccidiosis. Proceedings of the 13th World Association for the Advancement of Veterinary Parasitology Conference, 7–11 August, Berlin, Germany, S6-5