J. Vet. Med. B **53,** 482–487 (2006)

ISSN 0931–1793

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Canine Isosporosis – Epidemiology of Field and Experimental Infections

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With 6 figures

Received for publication March 31, 2006

Summary

Isospora spp. are the causative agents of canine isosporosis. Of the 3590 diagnostic samples from Austrian dogs (≤2 years old), 8.7% contained *Isospora* oocysts, 78% of which from dogs up to 4 months of age. Non-haemorrhagic and haemorrhagic diarrhoea were significantly more prevalent in *Isospora*-infected animals than in coccidia-negative ones. Twelve of 15 litters from a large commercial dog breeding unit (examined from the third to the 10th week of life) also excreted *Isospora* (average prevalence: 36.4%) in intensities from 333 to 35 000 oocysts per gram of faeces (opg). In experimental trials 26 3-week-old Beagle puppies were infected with low (600–6000), medium (10 000) or high (20 000) dose of *Isospora ohioensis*-group or *Isospora canis* field isolates. Additionally 21 puppies were infected as above and treated with a symmetrical triazintrione. Parasitological and clinical parameters were examined. The two *Isospora* species differed significantly concerning intensity and duration of excretion. The pre-patent period was 6–7 days for *I. ohioensis* and 10–12 days for *I. canis*. The latter species showed significantly longer excretion and higher opg. This was not influenced by simultaneous infections with both species. Individual patterns of faecal consistency were very variable, irrespective of the infection dose. Treatment significantly reduced both the intensity and the duration of oocysts excretion as well as diarrhoea in comparison with the infected, untreated group and thus proved to be effective against coccidiosis in experimental infections.

Introduction

Isosporosis is an ubiquitous protozoic infection of dogs all over the world. Three species of canine *Isospora* (syn. *Cystoisopora*) have been described, *I. canis*, *I. ohioensis* and *I. burrowsi*, and all three can cause diarrhoea in puppies (Pellérdy, 1974). Once the animals have overcome the infection they can develop immunity in varying degrees (Tenter and Deplazes, 2006). The long survival of the oocysts (Eckert et al., 2005) and their high ability to survive disinfection (Barutzki et al., 1981) support the maintenance of infection in canine populations. The parasites cause gross epithelial lesions in the distal parts of the small intestine. Petechiae and ulcerations of various sizes can appear (Pellérdy, 1974). Infections without symptoms of diarrhoea have been described, particularly in infections with *I. ohioensis* (Seeliger, 1999), but serious clinical illness and even death of infected puppies may occur (Daugschies et al., 2000). Seeliger (1999) noted changes in the faecal consistency of all shedding animals together with depression of behaviour in the field. Infections with *I. canis* were always accompanied with symptoms (Seeliger, 1999). The pathogenicity of *I. canis* is caused by its development in the deeper layers of the intestinal walls, unlike *I. ohioensis* which infects the cells of the lamina propria (Dubey, 1978a,b), and in the number of cycles of asexual development – *I. canis* undergoes three schizogonies (Lepp and Todd, 1974). Studies have demonstrated no differences in dog breeds, but the risk of infection appears to be higher in breeding units or kennels (Gothe and Reichler, 1990). Former studies describe different outcomes of infection. *I. ohioensis* can cause infections without symptoms (Seeliger, 1999) compared to experimental cases with a mortality of 66% of six infected animals – the remaining two were treated to avoid death (Daugschies et al., 2000). Clinical signs after experimental infections with *I. canis*, like increased body temperature, apathia and kachexia, were much more distinctive than after *I. ohioensis* infections (Becker et al., 1981). The aim of this study was to investigate the epidemiology, infection dynamics and clinical outcome of *Isospora* infections of puppies under field and experimental conditions and possible means of control.

Materials and Methods

Evaluation of *Isospora* prevalences in diagnostic samples

In a retrospective study results from 3590 faecal samples of dogs submitted to the Institute of Parasitology and Zoology (University of Veterinary Medicine Vienna) for routine diagnostic investigations between 1990 and 2005 were evaluated regarding the prevalence of *Isospora* spp. and other intestinal parasites. Only dogs younger than 2 years were included in this survey.

Epidemiology of Isospora in a commercial breeding unit

Fifteen litters from a commercial dog breeding unit were examined for enteric parasites. Pooled faecal samples of the puppies were collected weekly from the third ($n = 8$ litters) or fourth ($n = 7$ litters) week of life (wol). The sampling period was 7 ($n = 10$ litters), 6 ($n = 14$ litters) or 5 weeks ($n = 1$ litter). Pooled samples were examined for the presence of *Isospora* spp. with flotation in a semi-quantitative manner (low: one to three oocysts, medium: four to six oocysts, high:

more than six oocysts per field of vision at 100 × magnification; the whole area under the cover slip was examined) and, if positive, counted with the Mc Master method (Bauer, 2006). Species differentiation was done according to oocyst size as described (Tenter and Deplazes, 2006). *Isospora ohioensis* and *I. burrowsi* (i. e. *I. ohioensis*-group) have small oocysts of 24 × 20 μm and 21 × 18 μm, respectively, while *I. canis* oocysts are considerably larger, measuring about 39 × 32 μm.

Experimental infections

Twenty-six 3-week-old Beagle puppies (from 11 litters) were infected with sporulated oocysts of *I. ohioensis*-group or *I. canis* field isolates, respectively (see below). The puppies were divided into three groups receiving either a low (600–6000, $n = 7$), medium (10 000, $n = 12$) or high (20 000, $n = 7$) infection dose. Three animals of group 'high' were simultaneously infected with both species.

The number of oocysts per gram of faeces (opg), faecal consistency, weight gain, behaviour and skin turgor were examined daily (see below), starting with the 5th day post infection (5 dpi).

Additionally 21 puppies (from 6 litters) were infected and treated with symmetrical triazintrione (STT) suspensions as follows:

A = treatment during patency: low monospecific infection dose; treatment with 20 mg STT/kg body weight (BW) 8 dpi ($n = 5$); B = treatment during pre-patency: medium monospecific infection dose, treatment with 20 mg STT/kg BW 4 dpi ($n = 9$); C = treatment during pre-patency: high mixed infection dose (20 000 oocysts each of *I. ohioensis*-group and *I. canis*), treatment with 40 mg STT/kg BW on 4 dpi, ($n = 7$).

Both infection and treatment were done in a randomized, blinded setup. Puppies in each litter were ranked according to body weights and randomized in blocks (representing the respective infection/treatment groups) using a computer-generated randomization list. That way groups were evenly distributed across the litters.

Beagle dams and their litters were housed in the Institute's animal facilities under conventional conditions and fed with commercial dog food (meal fed twice a day) and water *ad libitum*. Puppies received starter food from the fourth wol. Litters were separated from each other to avoid cross-infection. Animals were marked individually by shaving. Rooms were cleaned daily and disinfected with Interkokask® (Inter Hygiene, Cuxhaven, Germany) before every new occupation. Staff was only allowed to enter with separate clothes and shoes. Dams were treated with an anthelmintic 2 weeks before and 2 weeks after birth, puppies 2 weeks after birth. Faeces of the dams were examined regularly for intestinal parasites, especially coccidia. During the trial animals were kept indoors at all times.

Infection

Infectious oocysts were obtained from field samples containing *Isospora* spp. oocysts after *in vitro* sporulation. Sporulated oocysts were stored in 2% potassium bichromate at 11°C for < 3 months prior to infection. Before use bichromate was removed by washing, oocysts were counted and individual doses were prepared and administered orally using a plastic pasteur pipet.

The day of infection was the 21st or 22nd day of life. An earlier time point proved to be unsuitable because the puppies cannot defecate without the dam's help before the fourth wol and therefore sampling during that time was extremely unreliable.

Sampling and diagnosis

Faecal samples were taken in the mornings on the day of infection and from 5 dpi for a period of 14 days. Faecal consistency, body weight, general behaviour and skin turgidity were used as clinical parameters. Faecal consistency was scored directly after collecting the samples as follows: faecal score (FS) 1 = firm, 2 = pasty, 3 = semi-liquid, 4 = liquid (FS > 2 was considered as diarrhoea). Body weight was determined weekly starting with the day of birth until the end of faecal sampling. General behaviour was scored as 1 = lively, 2 = reduced, 3 = apathic and 4 = comatous and skin turgidity was scored as 1 = normal, 2 = reduced, 3 = exsiccotic.

Oocysts per gram of faeces (opg) were determined by McMaster counting using a sugar salt flotation solution (Bauer, 2006).

On the first day of sampling (5 dpi) pooled faecal samples from each litter were examined for bacterial (*Escherichia coli*, *Salmonella* spp. *Clostridium perfringens*) and viral (distemper, parvo-, coronavirus) pathogens at the Institute of Bacteriology and the Institute of Virology (University of Veterinary Medicine, Vienna).

Statistical evaluation

Statistical evaluation was performed using the SPSS software package (version 11.5) or EXCEL (version 2002) for descriptive statistics, using the Student's *t*-test and chi-squared test to test for significant differences in oocyst excretion [excretion prevalence, mean Ln(opg + 1)] and faecal consistency (number of diarrhoea days). Correlation coefficients were calculated according to Spearman.

Results

Field infections with *Isospora* spp.

Of the 3590 faecal samples from dogs up to 2 years submitted to the Institute in a period of 15 years, 68.1% were negative, 8.7% contained *Isospora* oocysts and 23.2% contained other intestinal parasites. *Isospora* spp. positive samples ($n = 314$) contained oocysts of *I. canis* in 28.6%, oocysts of *I. ohioensis* in 53.5% and oocysts of both species in 17.8% of the cases. The other 831 positive samples contained other protozoa (53.5%), mostly *Giardia*, or helminths (46.5%), mostly *Toxocara* (Fig. 1). Within the 314 *Isospora*-positive samples 33.7% also contained other parasites, mostly *Giardia* (41.5%) or *Toxocara* (42.45%), less frequently other protozoa (10.3%) or cestodes (5.6%).

Of the 980 animals where the exact age was known (including 312 which were *Isospora*-positive), 59.5% were positive for parasites in the first 4 months of life, 41.8% with *Isospora* spp. and 51.1% with other species. Most *Isospora*-infections (78.0%) were found in the first 4 months of life, while dogs older than 1 year only rarely (1%) shed oocysts.

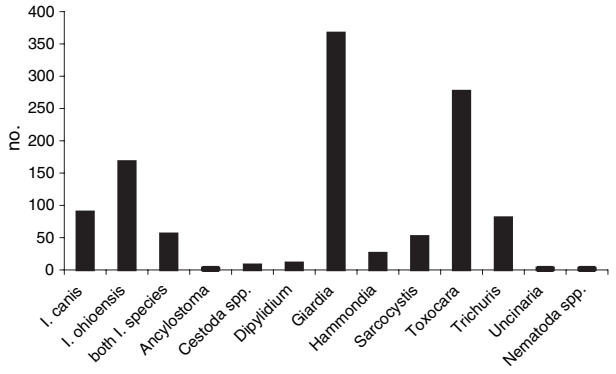

Fig. 1. Retrospective study on the distribution of parasites in positive diagnostic dog samples ($n = 1145$).

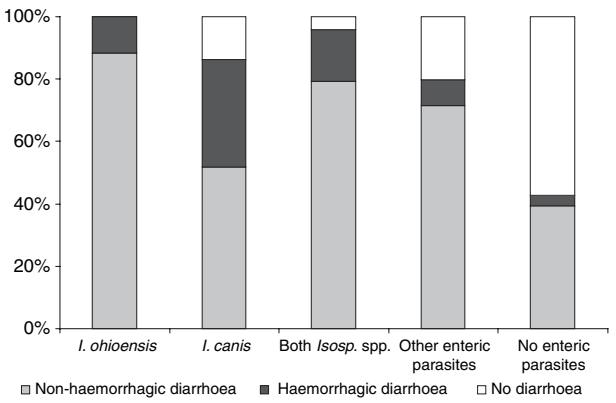

Fig. 2. Distribution of diarrhoea in diagnostic samples from clinical cases with or without isosporosis or other endoparasitic infections ($n = 359$).

In 359 cases (including 57 positive for *Isospora* and 160 for other enteric parasites) clinical reports were available. Diarrhoea was present in 77.2% of these cases; mostly non-haemorrhagic diarrhoea (NHD) in 66.6%, less frequently haemorrhagic diarrhoea (HD) in 10.6%.

All animals with *I. ohioensis* and 86.2% with *I. canis* had diarrhoea (11.5% and 34.5% HD, respectively) compared with 42.7% of the parasite-free animals (3.6% HD); 23.9% of the cases of NHD, 44.7% of the cases of HD and 6.1% non-diarrhoea cases were positive for *Isospora* (Fig. 2). Compared with parasite-free animals, *Isospora*-infected dogs had significantly more often NHD ($P = 0.000$) or HD ($P = 0.003$). This was also the case for coccidiosis versus infections with other enteric parasites ($P < 0.001$ for NHD and $P = 0.002$ for HD). Comparison between parasite-free animals and those with parasites other than *Isospora* shows that both NHD and HD were significantly more often seen in parasitized animals ($P < 0.001$ and $P = 0.002$, respectively).

Epidemiology in a commercial breeding unit

Ninety-nine samples from 15 litters were examined, 36 of which contained oocysts. Twelve litters were positive at least once. The onset of excretion ranged from the first to the last week of sampling [third wol: three of eight litters (37.5%),

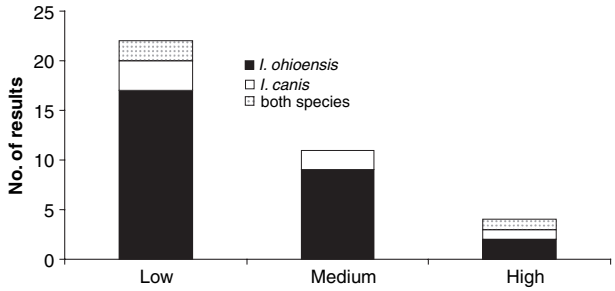

Fig. 3. Semi-quantitative evaluation of excretion intensity and species distribution in the positive faecal samples from the dog breeding unit. Low: one to three oocysts; medium: four to six oocysts; high: more than six oocysts per field of vision.

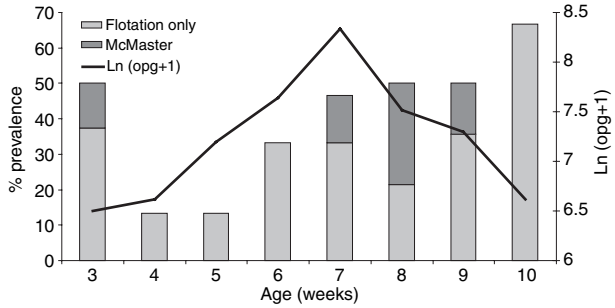

Fig. 4. Course of oocysts excretion prevalence (in %), excretion rates and intensity [given as mean Ln(opg + 1)] in the samples from a commercial dog breeding unit ($n = 99$ samples from 15 litters at 3–10 weeks of age).

fourth wol: two of 15 litters (13.3%), fifth wol: two of 15 litters (13.3%), sixth wol: five of 15 litters (33.3%), seventh wol: five of 15 litters (33.3%), eighth wol: four of 14 litters (28.6%), ninth wol: two of 14 litters (14.3%), 10th wol: one of three litters (33.3%)]. Most samples contained *I. ohioensis*, mostly in low to medium numbers (Fig. 3). Consequently, only 27 of the 36 flotation positive samples were detected by the McMaster method, including two *I. canis*-positive samples from the same litter. The minimum opg was 333, the maximum 35 000 [Ln(opg + 1) = 5.8–10.4] with a mean opg of 1000 and a mean Ln(opg + 1) of 6.9. Excretion prevalences were fluctuating over time (13.3–66.7%), as did the mean Ln(opg + 1) during the course of sampling which ranged from 0.9 to 4.4 (average of all animals in that wol) and between 6.5 and 8.3 for the positive puppies with a peak in the seventh wol (Fig. 4).

Experimental infection

Oocyst excretion

Regardless of the infection dose all untreated animals ($n = 26$) shed oocysts in a similar pattern (Fig. 5). The pre-patency of *I. ohioensis* was 6–7 days. Excretion persisted for 2–7 days with a mean Ln(opg + 1) of 1.6–9.4. The *I. ohioensis* peaked 7 dpi with Ln(opg + 1) = 9.4 and again 14 dpi with Ln(opg + 1) = 3.0. The pre-patent period of *I. canis* was 10–12 days and excretion lasted 10–11 days with a mean Ln(opg + 1) of 0.4–10.1. The excretion patterns were not influenced by double infections with both *Isospora* species. The excretion had one peak 12 dpi with Ln(opg + 1) = 10.1. The excretion of oocysts [given as the average Ln(opg + 1)] was

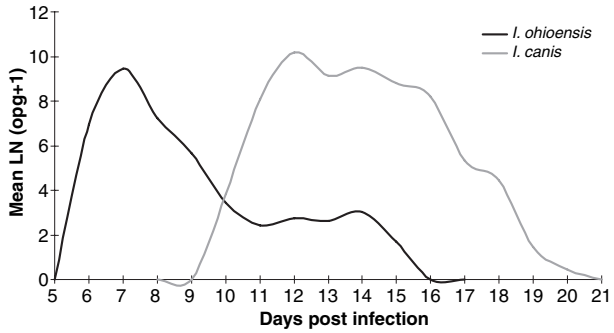

Fig. 5. Prevalences of oocyst excretion after experimental infection with *I. canis* ($n = 18$) or *I. ohioensis* ($n = 18$).

significantly higher for *I. canis* ($P = 0.007$). The number of excretion days was also significantly higher for *I. canis* ($P = 0.012$) than for *I. ohioensis*. Changes in behaviour and skin turgidity were not observed. The infection dose did not significantly influence the prevalence or the intensity of excretion ($P = 0.093$–1.0), nor did a double infection with both species ($P = 0.255$–0.381).

There was no correlation between infection dose and diarrhoea days ($r_S = 0.106$) or infection dose and opg ($r_S = 0.329$).

Faecal consistency

Mean FS ranged from 1.34 to 2.35 on different sampling days (average: 1.8 in the low, 1.7 in the medium and 1.6 in the high infection dose group). There was no significant correlation between the faecal consistency and the infection dose ($r_S = -0.263$; $P = 0.48$).

Treatment

Treatment significantly ($P = 0.001$) reduced the numbers of excretion days compared with untreated animals. The mean Ln(opg + 1) of groups B and C were significantly ($P < 0.000$) reduced compared with the untreated animals. All animals of group A were already excreting on the day of treatment and therefore the effect of treatment was less pronounced ($P = 0.059$). From 9 dpi (i.e. one day after treatment) three out of five animals in that group were negative while two continued to excrete oocysts. Four out of six animals of group B were positive for 1 day. None of the animals of group C ($n = 7$) excreted oocysts at any time (see Fig. 6a and b).

Most of the treated animals had no diarrhoea at any time. Mean FS on the different days reached from 1.4 to 2.25 in group A (average 1.9), 1.12–2.1 in group B (average 1.3) and 1.0–1.4 in group C (average 1.1). The mean number of days with diarrhoea (FS > 2) was statistically significantly higher ($P = 0.031$) in animals without treatment compared with all treated ones. A and B did not differ significantly from the untreated animals ($P = 1.000$ in both cases), while animals in group C had significantly fewer diarrhoea days ($P = 0.032$).

Weight development

Puppies had a birth weight of 240–450 g (average: 307 g) with the exception of one puppy that weighed 190 g. Weights in the last week of the experiment ranged between 1360 and 2705 g

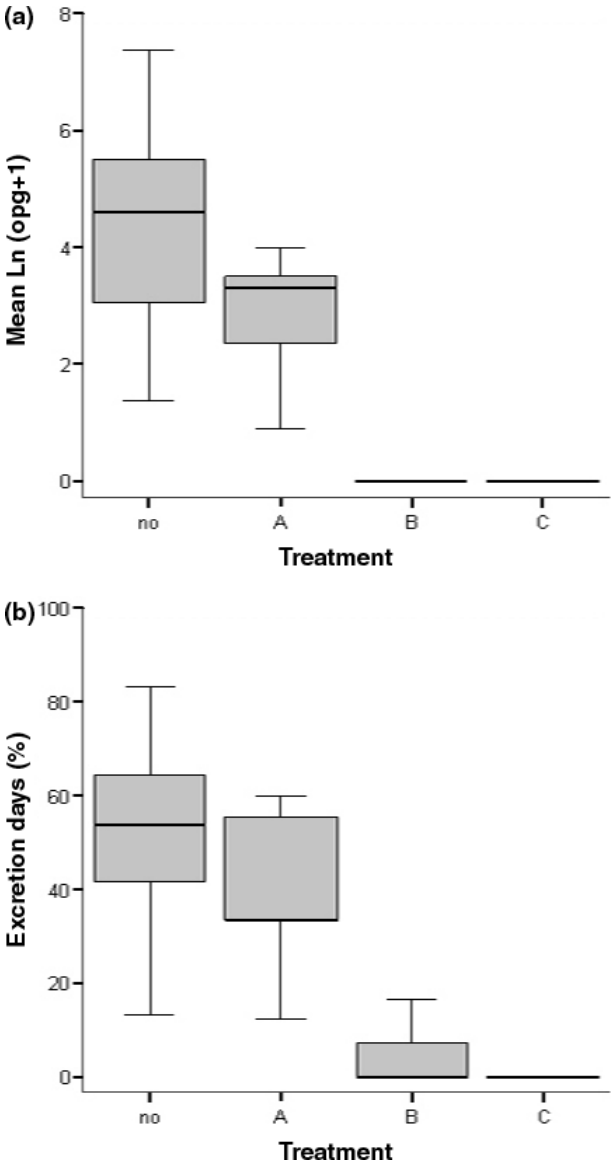

Fig. 6. (a) Median opg (and quartiles) in different triazinone treatment groups. 'no' = untreated ($n = 26$), A = treatment 8 dpi, 20 mg/kg ($n = 5$), B = treatment 4 dpi, 20 mg/kg ($n = 9$), C = treatment 4 dpi, 40 mg/kg ($n = 7$). (b) Percentage of excretion days in relation to sampling days in the different STT treatment groups.

(average: 1871 g). Regardless of the infection dose, the parasite species or treatment, all animals showed a stagnation of weight gain between the third and fourth wol.

Infection dose and weight gain from the third to the fourth wol (pre-patent period) were negatively correlated ($r_S = -0.599$; $P = 0.001$).

Differential diagnosis

Escherichia coli including haemolytic serotypes were present in all litters examined. Viral infections were not detected.

Discussion

Isospora infections are common in puppies and young dogs, and as expected the parasites were frequently diagnosed

both in the retrospective evaluation of diagnostic samples and in the investigated dog breeding unit. As described previously, the prevalences in dog breeding units reach 80% and more with high excretion intensities (Gothe and Reichler, 1990; Penzhorn et al., 1992; Bode, 1999). Infections are usually acquired during the suckling period (Hinaidy, 1991), although we could not determine an exact pattern of parasite acquisition in the field, as excretion was already present in the youngest age group (third wol) and could still be found in puppies shortly before weaning. Intensities appeared to peak around the seventh wol. Although indirect parasite transmission via paratenic hosts such as rodents is described (Dubey, 1975) oocysts from the environment must be considered as the primary source of infections in suckling puppies. The dimension of spreading is influenced by seasonal effects and management conditions (Bode, 1999). Little is known about age-related pathogenicity of canine coccidia, and reports on clinical outcome of infections range from asymptomatic (mainly in field infections with *I. ohioensis*) or symptoms like reduced weight gain and diarrhoea or even death, which have been associated with *I. canis* infections (Becker et al., 1981; Seeliger, 1999; Daugschies et al., 2000). A high incidence of diarrhoea in infected animals was recorded in the diagnostic samples from our Institute, but there may have been a strong bias towards the submission of samples from clinically ill patients for differential diagnosis. Generally, animals with enteric parasites showed significantly higher prevalences of NHD and HD in comparison to negative ones, but *Isospora*-infected dogs had more NHD and HD than other animals, whether these were infected with other intestinal parasites or not, indicating a strong correlation between coccidiosis and diarrhoea.

For the evaluation of the effects of controlled infection we established experimental infections using monospecific field isolates of both *I. canis* and *I. ohioensis*-group in young puppies. In contrast to previous reports the clinical signs of infection were limited irrespective of the infection dose or species. Only mild diarrhoea was found as a sign of pathophysiological changes in the gut during the pre-patent stage of infection. There was a negative correlation between infection dose and weight gain from the infection week (third wol) to the fourth wol indicating a negative influence of parasite infection on intestinal absorption; however, the general stagnation of weight gain during that period was probably caused by nutritional factors, e.g. changes from milk to more solid food. It can be speculated that either different isolates may vary greatly in virulence or that other pathogens must also be held responsible for the pathological and clinical changes during coccidiosis. Like other coccidial infections *Isospora* causes massive desquamation of the tips of the villi and cells of lamina propria of the small intestine (Dubey, 1978a,b) which can lead to clinical signs such as malnutrition and diarrhoea.

Oocyst excretion proved to be the most reliable parameter of infection, as described for *Isospora suis* infections in pigs (Mundt et al., 2006), although a dose-excretion correlation was not observed. The excretion pattern was not influenced by double infection.

Isospora canis was the more prolific of the two species with longer and higher excretion, which is probably because of the extra schizogony stage (Becker et al., 1981).

As it has been shown in previous studies (Daugschies et al., 2000; Lloyd and Smith, 2001) the treatment with triazinone had a marked effect on the clinical and parasitological outcome of infection. Diarrhoea or loose faeces were significantly reduced in treated animals. After treatment in the patent stage of infection excretion was reduced but not completely prevented (two of five animals continued to shed low numbers of parasites for 3 and 6 days, respectively), while an early application of the drug (before the onset of oocyst shedding) reduced excretion to single days (group B), and this may indicate that those samples probably represented passing oocysts ingested from infected but untreated litter mates in the randomized experimental setup rather than infection, as excretion usually lasted longer than 1 day in untreated animals. The coccidiocidal mode of action and efficacy of STT against all intracellular developmental stages of coccidia prevents the completion of the life cycle and consequently the shedding of oocysts. However, if treatment is carried out after the onset of oocyst excretion in the faeces the effect on the intracellular stages is masked by this excretion. In any case, early treatment provides the maximum control of the parasite. In the high dose group (C) excretion was not seen at all.

Under conditions that favour the spread of *Isospora* and infection at an early age such as high host density (commercial breeding kennels or animals shelters) or poor hygiene, systematic treatment of the puppies should be undertaken to control clinical disease and oocyst excretion. The latter point has often been neglected in the past when control of coccidiosis was attempted, but due to the environmental resistance of the oocysts reduction of contamination levels by prevention of excretion must be considered by far the most effective way to interrupt the life cycle, especially in surroundings where steam disinfection or application of chemical disinfection with effect on coccidia oocysts cannot be applied.

Acknowledgements

For the experimental part the dams and their litters were supplied by the Institute of Nutrition, University of Veterinary Medicine Vienna. The project leader received permission for animal experimentations according to national legal requirements (GZ 68.205/0153-BrGT/2005; GZ 68.205/174-BrGT/2004; GZ 68.205/0061-BrGT/2005).

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