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EFFICACY OF SOME ANTICOCCIDIAL DRUGS FOR TREATING COCCIDIAL ENTERITIS OF THE COMMON CARP CAUSED BY *GOUSSIA CARPELLI* (APICOMPLEXA: EIMERIIDAE)

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In this study, nine anticoccidial drugs commonly used in poultry were tested for efficacy for the prevention and treatment of *Goussia carpelli* (Apicomplexa) infection in common carp (*Cyprinus carpio* L.). To establish experimental infection with *G. carpelli,* paratenic host oligochaetes of the genera *Tubifex* and *Limnodrilus* were infected with oocysts, and laboratory-cultured parasite-free common carp fingerlings were infected by feeding to them oligochaetes containing sporozoites. The anticoccidial drugs (amprolium, narasin, maduramicin, salinomycin Na, lasalocid Na, diclazuril, robenidine HCl, monensin Na and toltrazuril), mixed in the food of the fish in a dose of 200 mg/kg, were fed for 12 days. Common carp fingerlings fed diclazuril, lasalocid, robenidine HCl or maduramicin and killed on day 14 after exposure were free from infection, while other groups treated with amprolium, toltrazuril, monensin Na, narasin or salinomycin Na harboured oocysts in the mucus and epithelium of the gut.

Key words: Coccidial enteritis, *Goussia carpelli*, common carp, anticoccidial drugs, efficacy, treatment

The pathological effects of fish coccidia infecting common carp represent a long known but relatively little studied field of fish pathology. On the occurrence and life cycle of one of these coccidia, *Goussia carpelli* (Léger et Stankovitch, 1921) numerous data can be found in textbooks (Schäperclaus, 1954; Kocylowski and Myaczynski, 1963; Molnár and Szakolczai, 1980; Bauer et al.,

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1981). These authors emphasise that 'diffuse coccidiosis' caused by *G. carpelli* is very common in common carp fingerlings and in older common carp, and in severe infection the enteritis caused by these coccidia results in emaciation and death of the fish. From the above-cited works and from other papers published on the subject (Schäperclaus, 1943; Ivasik and Kulakovskaya, 1959; Zaika and Kheisin, 1959; Musselius et al., 1965*a*; Jendrysek, 1993) it is not clear whether *G. carpelli* can cause coccidiosis in numerous fish species or this is a specific disease of the common carp. Several authors (e.g. Shulman and Zaika, 1964; Shulman, 1984) have suggested that this parasite can be found in numerous fish species and not only in cyprinids, while other researchers (e.g. Zmerzlaya, 1964; Kent and Hedrick, 1985; Lukeš et al., 1991; Belova and Krylov, 2000) speak of a very strict host specificity confined to the most closely related fish species.

Even less data are available on the possibilities of controlling this parasite. Most authors (Schäperclaus, 1954; Molnár and Szakolczai, 1980; Bauer et al., 1981) recommend freezing of the ponds, which is actually an effective method, since fish coccidia possess much less resistant oocysts than those of terrestrial animals. At the same time, the literature contains only few data on whether or not infected fish can be cured. Naumova and Kanaev (1962) reported that the feeding of Osarsol (acetarsone) was effective against *G. carpelli* infection. Musselius et al. (1965*b*) recommended the feeding of furazolidone. The first authors experimenting with modern anticoccidials were Kocylowski et al. (1976), who achieved good results with the feeding of amprolium chloride. Hine (1975) reported success with the use of Furanace while Solangi and Overstreet (1980) with that of monensin Na. As these drugs were not tested extensively, Bauer et al. (1981) continued to regard the freezing and drying out of ponds and their disinfection with chloride of lime as the most effective method. The implementation of exact laboratory experiments became possible only after Molnár (1979) had recognised that intensive infection with coccidia could be established only through the involvement of paratenic hosts. By this method, Paterson and Desser (1982) produced intensive infection in fathead minnow and common shiner with *Goussia iroquoina* while Steinhagen and Körting (1990) in common carp with *G. carpelli*. Molnár et al. (2005) have studied the host specificity of *G. carpelli* by the same approach, demonstrating that this species is a strictly species-specific parasite of the common carp.

For the above reasons, it would be essential to survey fish coccidiosis as an important disease and to develop an effective method for its control and treatment. The recent expansion of the relevant knowledge enables us to study fish coccidiosis under laboratory conditions, using experimental methods.

In the experiments reported in this paper, the efficacy of nine anticoccidial agents widely used in Hungary against poultry coccidiosis was tested for the control of *G. carpelli* infection experimentally induced in infection-free common carp.

Materials and methods

Goussia carpelli oocysts used for the experimental infection were obtained from the gut of common carp brought from fish farms and fasted for a few days. To isolate oocysts, the fish were killed and oocyst-containing mucus samples, free of gross faecal particles, were stripped off the epithelial layer of the intestinal mucosa. Subsequently, the oocysts were mixed into the mud in dishes containing the oligochaetes *Tubifex tubifex* and *Limnodrilus hoffmeisteri* reared free of infection in the laboratory, and then the experimental fish were fed oligochaetes infected 17–30 days earlier. According to our previous findings (Molnár et al., 2005), through their constant mud-eating the oligochaetes exposed to oocysts for at least 10 days ingest oocysts in an amount sufficient for the accumulation of a sporozoite quantity necessary for successful infection. Three to 5 cm long common carp fingerlings used for the experiment were hatched in the laboratory and fed parasite-free food (brine shrimp, frozen plankton and dry common carp food). Three days before the experiments, the fish selected for experimental infection were placed into plastic dishes containing approximately 0.3 l of water, one by one. In addition to the usual premix, the fish were also given infection-free tubifex by way of adaptation. On the day of infection, the tubifex specimens containing sporozoites were cut into small, approx. 1-mm slices and the slices were mixed so as to establish uniform infection in the fish. The fish rapidly and consistently ate the tubifex slices. After the infection, the fish were kept in isolation for some hours, and then the fish kept for the same experimental purpose were placed into a common dish and kept there until killed.

As the fish were infected one by one and kept individually in the different experiments, maximum 5 fish specimens were treated with each of the anticoccidials tested. Another reason for using such a relatively low number of fish was that diagnosing the infection by thorough examination of the entire gut of fish after killing took a rather long time. Nine anticoccidials most commonly used in Hungary were selected for the experiments (Table 1).

The medicated food contained some of the anticoccidial drugs (amprolium, salinomycin Na, monensin Na, toltrazuril) in doses recommended against diseases caused by other fish-parasitic protozoans (*Ichthyophthirius*, *Hexamita*) or myxozoans (*Myxobolus*) (Tojo and Santamarina, 1998, 2001; Shinn et al., 2003; Athanassopoulou et al., 2004). For the sake of standardisation we used similar doses of the other tested drugs as well, which meant that in the case of diclazuril, maduramicin and robenidine significantly larger amounts of drugs were mixed in the food than recommended by the manufacturers for treating chicken coccidiosis (see Table 1). From the pure active ingredients and from the premixes used for the feeding of fish we prepared a medicated food which contained 200 mg of the pure active ingredient per kg of food. This practically meant medicated foods of 0.02% active ingredient concentration. According to

the observations made in previous feeding experiments, we expected that at the temperature used in the experiment (22–24 °C) the fish would consume food in a quantity corresponding to at least 5% of their body weight every day.

Table 1

Active ingredients, trade names and manufacturers of anticoccidial agents tested for efficacy for the control of diffuse coccidiosis caused by *Goussia carpelli*

Active ingredient	Trade name	Manufacturer	Doses recommended for chickens (mg/kg of feed)
Amprolium	Amprolium	Merial	62.5–125
Narasin	Monteban-100	Eli Lilly & Elanco	60–70
Maduramicin	Cygro 1%	Alpharma AH	5
Salinomycin Na	BIO-COX 120	Alpharma AH	50–70
Lasalocid Na	Avatec 15%	Alpharma AH	82.5
Diclazuril	Clinacox	Janssen Pharmaceutica	1
Robenidine HCl	Cycostat® 6.6%	Alpharma AH	33
Monensin Na	Elancoban 200	Eli Lilly & Elanco	100–120
Toltrazuril	Baycox 5%	Bayer AG	25

The anticoccidial drugs or the premixes were pulverised in a braying mortar and mixed with a small quantity of finely powdered food, and then the resulting mixture was added to the whole amount of food by thorough mixing. Toltrazuril was an exception: this compound was first diluted with water, then the resulting solution was sprayed onto the food and mixed thoroughly. Subsequently vegetable oil was added to solidify the food sufficiently so that it could preserve its form for a long time after having been placed into the water.

Feeding of the medicated food started immediately after the fish had ingested the tubifex pieces used for infection. The food was fed once a day, in the morning hours. Any food remnants left over in the dish were sucked out in the afternoon, and occasionally a complete change of water was performed.

The medicated food was fed for 12 days. Subsequently the fish were fasted for two days to allow their gut to empty, and then they were killed on day 14. According to the results of our previous studies, this is the day when the chances of detecting any oocysts produced are the highest.

After cutting open the abdominal wall of fish killed by decapitation, the intestines were separated from the other internal organs. The gut was opened in longitudinal direction, and the mucus drawn off the foregut and midgut and placed under a coverslip was examined by microscopy at 200- to 400-fold magnification. The intestinal wall was also examined by squashing a segment of the gut between two slides and studying it under microscope at 100-fold magnification. The numbers of both the sporulated and the unsporulated oocysts were recorded. The severity of infection was scored as follows: +: only 1–9 oocysts

were present in the scrapings; ++: presence of 10–30 oocysts in the entire gut; +++: presence of 5–10 oocysts per visual field; ++++: presence of more than 10 oocysts per visual field; +++++: massive infection of the gut, with the presence of numerous oocysts in the intestinal mucus and masses of oocysts in the gut wall as well.

Results

The fish readily consumed all medicated food mixtures every day in a quantity corresponding to about 7% of their body weight in average. Mortality attributable to drug feeding did not occur during the experiments; however, death of a few fish attributable to mechanical injury during the cleaning operations was occasionally recorded.

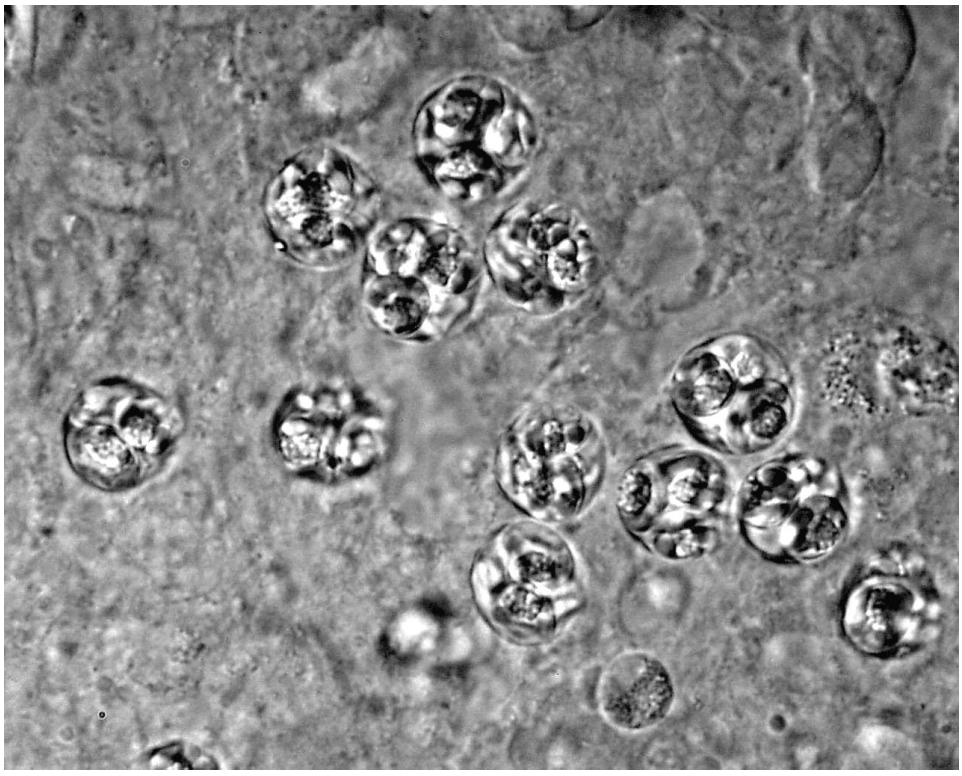

Fig. 1. Sporulated oocysts of *Goussia carpelli* in the mucus drawn off the gut mucosa of common carp. Fresh mount, × 1500

The sporulated oocysts of *G. carpelli* were easily recognisable in the mucus and epithelial scrapings free from coarse intestinal content by their typical

round shape and the yellow bodies usually surrounding them (Fig. 1). Only in 6 out of the 11 infection experiments presented in Table 2 could we produce infection when all controls became infected and when oocyst masses similar to those shown in Fig. 2 could be detected in the gut wall of the fish. In these cases (i.e. in experiments 1, 3, 5, 7, 8, 9 and 11), the intensive infection produced in the control fish allowed us to obtain reliable data on the efficacy of the anticoccidials studied, while the observations made in the other cases (in experiments 2, 4, 6 and 10) can only be regarded as results indicating trends. On the basis of the 11 experiments, we can state that the preventive feeding of four anticoccidials (diclazuril, lasalocid Na, robenidine HCl, maduramicin) at the doses applied consistently prevented the development of *G. carpelli* infection, while the feeding of the other five drugs (amprolium, toltrazuril, monensin Na, narasin and salinomycin Na) proved to be ineffective or of low efficacy. The partial efficacy of the latter drugs, especially that of salinomycin Na and monensin Na, was suggested by the observation that the infection developing in the groups medicated with these agents was consistently of lower degree than that found in the control groups.

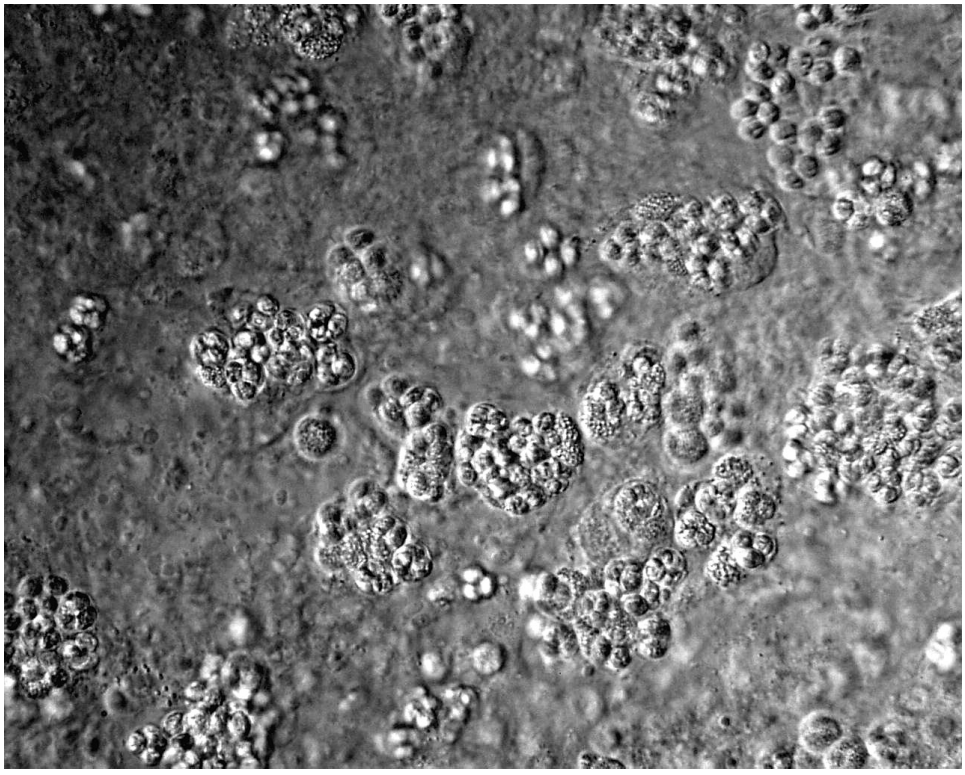

Fig. 2. Masses of *Goussia carpelli* oocysts in the gut wall of an infected control common carp. The gut wall was squashed between two glass slides. Fresh mount, × 500

Table 2

Efficacy of anticoccidial drugs mixed in the fish food in a dose of 200 mg/kg against *Goussia carpelli* coccidiosis experimentally produced in common carp fingerlings

Anticoccidial drug	Serial number of experiment										
	1	2	3	4	5	6	7	8	9	10	11
Amprolium	5/2 ++ +	5/3 + + +	5/3 +++, +++, ++						6/6 ++, +, ++, +, +, +	5/2 ++++, +++	
Toltrazuril	5/2 + +	5/3 + + +	4/4 ++++, ++++, +++, +++				5/5 +++, +, +++, ++, ++		5/5 +++, ++, +, +, +	5/1 ++	
Monensin									5/4 +, +, +, +, +	5/0	5/3 ++, ++, +
Narasin		5/2 +, +	4/2 ++, ++	5/0	5/1 +++	5/1 +		5/3 +++, +++, +++	5/5 ++++, +++, ++, ++, ++	5/0	5/2 +, +
Salinomycin				5/0	5/3 +, +, +	5/4 +, +, +, +			5/4 +, +, +, +	5/0	5/3 +, +, +
Diclazuril							5/0		5/0	5/0	5/0
Lasalocid				5/0	5/0	4/0			5/0	5/0	5/0
Robenidine							5/0	5/0	5/0	5/0	5/0
Maduramicin				5/0	5/0	5/0		5/0	4/0	5/0	4/0
Control	5/5 ++ ++ ++ ++ ++	5/3 + + +	5/5 +++, +++, ++, ++, ++	5/1 +	5/5 +++++, +++++, ++++, ++, +	5/3 ++, +, +	4/4 +++++, +++++, +++++, +++++	5/5 +++++, +++++, +++++, +++++, +++++	5/5 ++++, ++++, ++++, ++++, ++++	5/3 ++++, +++, +++	5/5 +++++, ++++, ++, +, ++,

Explanation of symbols: +: 1–9 oocysts in the gut; ++: 10–30 oocysts in the gut; +++: 5–10 oocysts per visual field at 200-fold magnification; ++++: more than 10 oocysts per visual field at 200-fold magnification; +++++: many oocysts in the intestinal mucus and in the gut wall; Numerator/denominator: number of fish included in the experiment/number of fish found infected

Discussion

Although coccidiosis is a long known and rather common disease of fish, little attempt has been made to treat this disease and to find a drug effective against it. Our results presented in this paper suggest that some of the anticoccidials widely used for the therapy and prevention of coccidioses in warm-blooded animals can be used successfully for the control and prevention of coccidial infections of fish as well. In our laboratory experiments, the preventive feeding of four anticoccidial agents (diclazuril, lasalocid Na, robenidine HCl, maduramicin) completely prevented the development of enteritis caused by *G. carpelli* and provided complete protection against it. The favourable results of the laboratory experiments suggest that these anticoccidials could be tested successfully also in fish pond environment and, thus, fish coccidioses so far untreatable with medicaments could become diseases easy to control. Our results are rather surprising, as in previous studies reported in the literature the effect of anticoccidials used against fish coccidioses, such as amprolium (Kocylowski et al., 1976) and monensin Na (Solangi and Overstreet, 1980), was difficult to assess, and the efficacy of narasin and salinomycin Na also manifested itself only in a reduced intensity of infection as compared to the control. The present experiments do not allow us to draw definite conclusions on the efficacy of the latter drugs, as the doses used in the test were relatively uniform, and they were rather different from doses recommended by manufacturers for treating coccidian infections of warm-blooded animals: the active ingredient concentration of the medicated foods was consistently 0.02%, and the best results were always obtained with the most overdosed drugs. In these preliminary experiments we did not attempt to study either the effect of increasing or decreasing the active ingredient concentration or the elutriation of anticoccidial agents from the medicated food. To warm-blooded animals, the anticoccidials under study are administered in the form of dry feed mixtures and, thus, the aqueous phase does not play a role in these animals. Therefore, when used in elevated doses or in a manner preventing their elutriation from the food, further agents may also prove suitable for use as anticoccidials in fish. The latter concern is especially valid for toltrazuril, since in the present study that drug was used in a water-soluble formulation lowering the chance of its uptake by the fish. Despite all these considerations, we feel that our preliminary results are promising and point towards the development of an effective method of fish coccidiosis control for pisciculture.

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