EFFICACY OF OXFENDAZOLE FOR THE TREATMENT OF GIARDIOSIS IN DOGS. EXPERIMENTS IN DOG BREEDING KENNELS

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Summary :

Giardiosis is one of the most frequent parasites of dogs and cats. Since several years, the treatment is based on the use of metronidazole. A coproscopic study in four dog kennels was conducted to demonstrate, at a significant level, the efficacy of oxfendazole (Dolthène®, Merial). At the posology of 11.3 mg/kg each day during three days (D1, D2 and D3), no dogs eliminated *Giardia* cysts and all dogs are clinically cured. The importance of hygienic measures is underlined. In kennels 1 and 2 where hygienic conditions were poor, dogs reexcreted cysts again after treatment. In kennels where the boxes were disinfected, no dogs, treated with 22.6 or 11.3 mg/kg, reexcreted *Giardia* cysts.

KEY WORDS : *Giardia duodenalis*, dog, oxfendazole.

Résumé : ÉFFICACITÉ DE L'OXFENDAZOLE DANS LE TRAITEMENT DE L'INFECTION DES CHIENS PAR *GIARDIA DUODENALIS*. ESSAIS EN ÉLEVAGES DE CHIENS

La giardiose est l'une des parasitoses digestives les plus fréquentes chez le chien ou le chat. Le traitement a longtemps reposé sur l'emploi du métronidazole. Un essai conduit dans quatre élevages de chiens démontre significativement l'efficacité de l'oxfendazole, Dolthène®. Employé à la posologie de 11,3 mg/kg pendant trois jours de suite (J1, J2 et J3), l'oxfendazole négative l'excrétion des kystes de Giardia duodenalis *et entraîne une guérison clinique. L'importance des mesures sanitaires de désinfection des box, empêchant les réinfections, est soulignée. Dans les deux élevages où les conditions hygiéniques sont médiocres, les chiens traités réexcrètent des kystes de protozoaires. Dans les élevages où des mesures de désinfection ont été mises en œuvre avant les traitements, aucun chien, traité à 22,6 ou 11,3 mg/kg, n'a réexcrété de kystes de* Giardia.

MOTS CLÉS : Giardia duodenalis, *chien, oxfendazole.*

INTRODUCTION

Giardiosis is an infectious disease of the small intestine due to *Giardia duodenalis* (= *Giardia intestinalis* = *G. lamblia)* clinically showing the development of an enteritis with chronic diarrhoea with steatorrhea (Barr & Bowman, 1994; Bourdeau, 1993; Leib & Zajac, 1999; Thompson & Reynoldson, 1997; Thompson *et al.*, 2000; Zajac, 1992). Infections are associated with a decrease of microvillus surface area, reduced intestinal enzyme activity and increased intestinal transit (Deselliers *et al*, 1997). These pathological changes result in a malabsorptive diarrhea, which is the major clinical sign of giardiosis. This protozoan is the most commonly identified pathogen of humans and animals (livestock and pets) throughout the world (Thompson *et al.*, 2000). Giardiosis is frequent in carnivorous companion animals, showing living sound carriers that form the parasite tank (Barr & Bowman, 1994; Bourdeau, 1993; Leib & Zajac, 1999; Thompson & Reynoldson, 1997; Zajac, 1992). Giardiosis is also of great importance in livestock and is frequently associated with diarrhea in dairy cattle (O'Handley, 2000). It is considered by several authors to be a zoonosis, even though various adjusted strains host species are genetically singled out (Capon, 1989; Gasser, 1990; Hopkins, 1997; Thompson *et al.*, 2000). Some strains seem to be able to infect human from livestock or dogs and vice versa, and, in the opposite, other strains seem to be very specific. Further genetic studies are needed to understand these variations (Gasser, 1990; Hopkins, 1997; Meloni *et al.*, 1995; Thompson *et al.*, 2000). Giardiosis is the most commonly non viral reported intestinal infection in humans. Domestic carnivores could represent a significant parasite source for their owners. This digestive parasite is underestimated by veterinarian practice because of the difficult diagnosis which is based on faeces coproscopy (Barr & coll., 1994; Beugnet, 1998; Beugnet, 1996). It is considered as a major cause of diarrhoea in dogs, in kennels or at home.

A study on urban domestic carnivores intestinal parasitism (Beugnet *et al.*, 2000) was led in Paris suburbs in 1998/1999. It showed that 14.7 % of cats were *Giardia duodenalis* infected. 30.4 % of dogs under six months-old were infected versus 7.1 % of adults. This

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flagellate seems to be one of the most frequent intestinal parasites in dogs and cats, as helminths (Franc *et al.*, 1997). A first study led by the Parasitology laboratory of the Lyon Vet School showed *Giardia* cysts in 10 % of the faeces by coproscopic studies conducted on carnivores suffering from diarrhoea and having gone to consulting (Beugnet, 1998).

Evolution is favourable after the implementation of a symptomatic and specific treatment. This latter has long been based on metronidazole (Flagyl®) oral use, at a posology of 20 mg/kg, twice a day during 10 days (Barr & Bowman, 1994; Zajac, 1992). Some experiments stated a poor efficacy and existing intolerance signs such as nausea, vomiting or ataxy (Thompson & Reynoldson, 1992). Metronidazole chemoresistant parasitic strains seem recrudescent in the field and could explain human medecine therapeutic failures (Johnson, 1993; Upcroft & Upcroft, 1997).

Benzimidazoles activity was demonstrated more recently with an efficacy above 90 % (Barr *et al.*, 1993; Barr *et al.*, 1994; Barr *et al.*, 1998; Morgan *et al.*, 1993). The use of these molecules could be favourable due to their tolerance, even at a high posology. Albendazole seems therefore effective when administrated to dogs at a posology of 25 mg/kg twice a day during two days (Barr *et al.*, 1993) while fenbendazole (Panacur 250®, Intervet) is also effective at 50 mg/kg during three days (Barr *et al.*, 1994). Oxfendazole (Dolthène®, Merial) is an anthelminthic used in several countries like France, Italy, Spain, or Germany for dogs deworming. Its potency on *Giardia duodenalis* may prove very interesting joint to the standard anthelminthic spectrum. Oxfendazole is one of the anthelmintic most often used in Europe to deworm the dogs. Febantel, fenbendazole, and oxfendazole are three benzimidazoles which are based on the same nuclear and which have the same metabolites after administration (Fig. 1) (Towsend & Wise, 1990). As fenbendazole is active against *Giardia*, it was of great interest to demonstrate the efficacy of oxfendazole (the active sulfoxyde metabolite of fenbendazole).

In this study, the trials were done by veterinary practitioners in dog kennels. A coproscopic screening helped to identify *Giardia* infected dogs and later to treat them. Both *Giardia* cysts excretion and these animals clinical state were followed up.

MATERIALS AND METHODS

Infected dogs come from five pup kennels dedicated to sale. A coproscopic study helped to identify *Giardia duodenalis* infected dogs. 199 dogs (73 males and 126 females) were added to the previous study. These dogs belong to various breeds: Scottish, Westie, Lhassa Apso, Poodle, American Cocker, Setter, Alsatian, Leonberg, and St Bernard.

Out of 199 dogs, 34 (18 males and 16 females) proved to be *Giardia duodenalis* infected. These were added to the treatment trials. 19 dogs were under one year old, and five above. Sex and age are mentioned in Table I for each dog.

Oxfendazole (Dolthène®, Merial Laboratories) is a liquid orally given at a posology of 11.3 or 22.6 mg/kg, three days running. A coproscopic follow-up shows the possible decrease of protozoan cysts in faeces. Treatment is carried out at D1, D2 and D3. Semi-quantitative coproscopic studies were made by MacMaster's technique at D0, D5, D7, D9 and D12. After experimental infection with *Giardia* cysts, the dogs excreted cysts in 48 to 72 hours (Leib & Zajac, 1999); so a survey during 10 days will show if dogs are cured or not. There is no description in dogs of long periods without excretion, and three coproscopies at 48 hours interval

Oxfendazole use at a posology of 11.3 mg/kg each day during three days

Kennel 1	Sex (M/F)	Age (month)	D0	D9	D12
Mean	6 dogs: 3M, 3F	4.67	5	0*	1.67*
Sem		2.58	0	0	0.52

U Rank test: significant difference beteween D0 and D12 with risk error of 10^{-4}

Oxfendazole use at a posology of 22.6 mg/kg, three days running

Kennels 1 and 2	Sex	Age (month)	D0	D5	D7	D9
Mean	11 dogs: 7M, 4F	3.81	3.64	2	0.82*	1.73*
Sem		2.08	1.57	2.05	0.87	1.19

U Rank test: significant difference beteween D0 and D9 with risk error of 2 %

Oxfendazole use at a posology of 22.6 mg/kg, three days running. Treatment in disinfected boxes.

Kennel 3	Sex	Age (month)	D0	D5	D7	D9
Mean	10 dogs: 5M, 5F	5	5	0*	0*	0*
Sem		0	0	0	0	0

U Rank test: significant difference at D5, D7 and D9 with D0 at less than 10^{-6}

Oxfendazole use at a posology of 11.3 mg/kg, three days running. Treatment in disinfected boxes.

Kennel 4	Sex	Age (month)	D0	D5	D7	D9
Mean	9 dogs: 4M, 5F	24.11	4.33	0*	0*	0*
Sem		21.47	1	0	0	0

U Rank test: significant difference at D5, D7 and D9 with D0 at less than 10^{-6}

Sem: standard error of the mean.

* Significant difference between the number of *Giardia* cysts at D0 compare to D5, D7 or D9.

Table I. – Results of the four successive experiments.

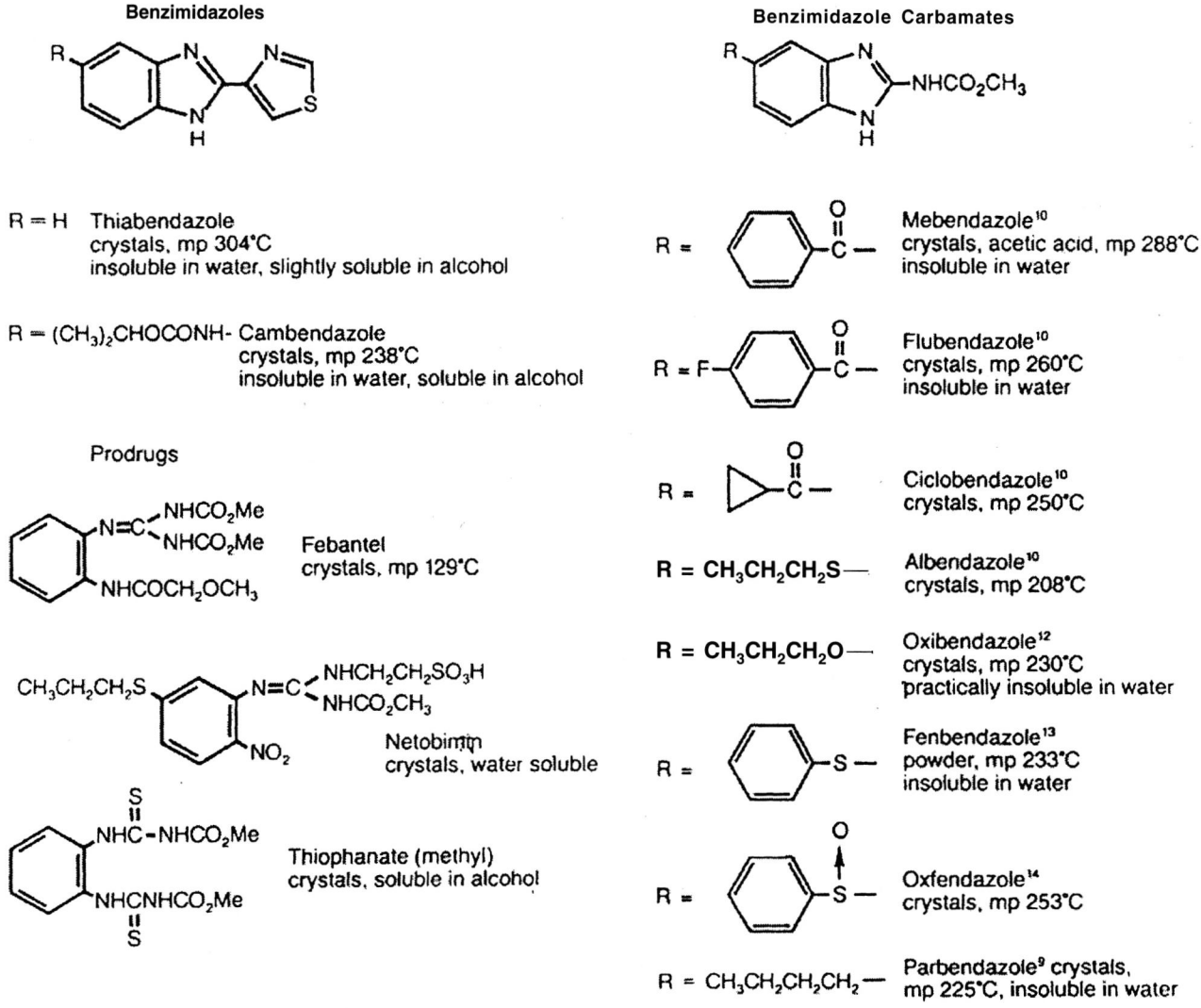

Fig. 1. – Chemical structures of some benzimidazoles anthelmintics (Towsend & Wise, 1990).

can diagnose giardiosis with a sensibility superior to 95 % (Barr *et al.*, 1992; Leib & Zajac, 1999).

The floating technique, using a high density liquid, especially suits protozoan cysts research, since these latter are not very dense (Barr *et al.*, 1992). Magnesium sulfate with 1.28 density up to 25° C (35 g of magnesium sulfate per 100 ml of water) was held. Cysts number quantification is given with an order of size. Quantification notions:

(5): more than 1,000 *Giardia* cysts /5 g faeces,
(4): between 200 and 1,000,
(3): between 100 and 200,
(2): between 10 and 100,
(1): presence: less than 10,
(0): no cysts.

Statistical comparisons have been made between the number of cysts excreted before and after the anthelmintic treatment using non parametric test (Mann & Whitney U test).

Four successive experiments were conducted. The first two in two kennels where hygienic conditions were poor and with no disinfection measures before treatment. The following two trials were carried out in two other kennels where dogs are in boxes with disinfection measures before treatment. The disinfection was preceded by a cleaning with high pressure water (using Karcher). Then the disinfection was made using both sodium hypochlorite solution and an ammonium IV solution for farms (TH4$^{+®}$, Sogeval Laboratory).

RESULTS

PRELIMINARY SURVEY

Out of the 199 dogs added to the survey, 136 (68.3 %) are at least infected by one parasite. *Giardia duodenalis* was found in 34 dogs

(17.1 %). This prevalence was increased to 44.45 % in dogs under six months (24/54).

Oxfendazole at a posology of 11.3 mg/kg, with no disinfection measures

At first, oxfendazole was orally given three days running (D1 to D3) at a posology of 11.3 mg/kg to all animals proved positive at screening.

In kennel 1, *Giardia* cysts were detected in six dogs including four pups under three months-old and two young aged eight months. All these pups had a diarrhoeic enteritis. Results are shown in Table I.

A decrease in the *Giardia* cysts number is underlined, alongside with negativation for all puppies at D9. In spite of a reexcretion of cysts on Day 12, the difference is significant at 10^{-4}. All pups showing digestive troubles stopped at D5, without later reappearance. However, kennel condition, with poor hygiene (animals in paddocks with grassy path from which floor disinfection is unfeasible), surely results in a fast new infection of pups. Since D12, a cysts reexcretion is noticed in lesser ratio.

The following trials were led according to two axes so as to cure it all: 22.6 mg/kg posology trial and health measures implementation to restrict reinfections by cysts.

Oxfendazole at a posology of 22.6 mg/kg, with no joint disinfection measures

Animals from kennel 1 that proved positive at the end of the first trial took part again in the trial with double posology, except dogs one up to four that were sold meanwhile. Other pups, suffering from diarrhoea and emaciation, and excreting *Giardia* cysts were included in this second trial. In kennel 2, five dogs were detected among which four pups under six months-old and one young adult aged eight months.

All these animals orally took oxfendazole (Dolthène®) at a posology of 22.6 mg/kg, three days running.

At double posology there is a significant decrease of the cysts number, from thousands per gram of faeces to none, since D5 for kennel 2. That is to say a 100 % efficacy at D5. This kennel had higher hygiene conditions than kennel 1. A clinical recovery is noticed on all pups.

In kennel 1, the cysts number decrease is less important, going from thousands to few hundreds. Reinfections or *Giardia* multiplication retakings occur from D7 and D9 according to experiments, that is to say two up to three days after the end of the treatment. In spite of the reexcretion of cysts, the difference between D0 and D7 counts is significant at less than $1\,10^{-3}$ and between D0 and D9 is significant at 2 %.

It appears to be necessary to carry out a supervised trial in which reinfections are restricted to a minimum by strict hygiene measures.

Oxfendazole at a posology of 22.6 mg/kg, with boxes disinfection

Boxes disinfection is done on the first day of treatment. Boxes washing followed by a disinfection with ammonium IV and/or sodium hypochlorite were made in kennels 3 and 4.

In kennel 3 with very good hygiene conditions (regular worming, daily boxes cleaning, animals breakdown by age groups, maternity placing for pregnant and nursing females) and after boxes disinfection on the first day of treatment, there is a 100 % efficacy on 10 pups with no excretion coming back. These 10 pups under five months-old (five males and five females) all suffered from a clinical giardiosis. All signs disappeared since D7, and never reappeared.

Oxfendazole at a posology of 11.3 mg/kg, with boxes disinfection

This experiment was carried out in kennel 4. Eight adults infected and a six months-old pup were detected. Boxes were washed and disinfected on the first day of treatment. As in the previous experiment, dogs did not go out of their boxes during the whole experiment (from D1 until D9) in order to avoid all reinfection risk. There is a stop of cysts excretion in 100 % of cases.

DISCUSSION

In the first experiment (kennel 1), dogs have been treated with oxfendazole at the posology used for anthelmintic treatment. Results showed a significant decrease in the number of cysts (> 95 %) but no disappearance of their excretion. There are two possible explanations: either the oxfendazole posology was not high enough to overthrow *Giardia*, or dogs got infected again between the end of the treatment and coproscopic studies. Reinfection assumption is plausible due to cysts resistance in the outside space (two months at 8° C; one month at 21° C; partial resistance at – 13° C during two weeks), and the fact that floor disinfection was not done because of kennel conditions (Barr & Bowman, 1994; Thompson & Reynoldson, 1993; Zajac, 1992). On the other hand, only one coproscopic study was carried out during screening, with the result that some carriers animals may not have been detected and therefore remained sources of cysts for dogs on test (Barr & Bowman, 1994; Barr *et al.*, 1992).

To meet these two assumptions a double dose compared to the previous one (22.6 mg/kg) was tested, setting up or not hygiene conditions (kennel premises disinfection).

In kennels 1 and 2, the double dose was used without floor disinfection (paddock, mud floor and grass kennels) and the efficacy of treatment remained partial (decrease of the emitted cysts number).

In kennel 3, the double dose of oxfendazole joint to floor disinfection helped achieve a total disappearance of cysts excretion and pups clinical recovery. This experiment underlines the importance of the dogs reinfection risk by cysts existing in their environment.

Fenbendazole is effective in the canine giardiasis treatment at the usual anthelminthic posologies and administration rates for dogs (Barr *et al.*, 1994; Zajac *et al.*, 1998; Zajac *et al.*, 1992). Oxfendazole should have been tested at anthelmintic posology (11.3 mg/kg) restricting reinfections. The experiment in kennel 4 proves this effect, since none of the nine treated dogs reexcreted cysts during the three running controls. Canine giardiosis treatment can be based on oxfendazole use at a posology of 11.3 mg/kg *per os* three days running.

Out of 34 treated dogs, at simple or double posology, none showed troubles or signs of toxicity of the molecule.

This study being led within dog kennels dedicated to sale, no breeder accepted untreated sample portion. Besides, all dogs – infested and showing clinical signs or not – were wormed. Several studies on canine giardiosis prove that dogs show no self-recovery inclination and that signs such as excretion can continue months and even years (Barr & Bowman, 1994; Thompson & Reynoldson, 1993). Giardiosis treatment has long been based on metronidazole use. Its potency is still not complete, undesirable effects are frequent, and chemoresistant *Giardia* strains are now described (Barr & Bowman, 1994; Jonhson, 1993; Upcroft & Upcroft, 1993). Some investigators have pointed out the potency of some anthelmintics from the benzimidazoles group, especially albendazole, fenbendazole or febantel. Albendazole is not used in dogs and presents a medullary toxicity in some cases (Meyer, 1998; Stokol, 1997). Febantel joint to pyrantel and praziquantel has some potency on *Giardia* when administrated three days running (Barr *et al.*, 1994; Zajac *et al.*, 1998), but it is usually used in the field with a unique administration. Fenbendazole is effective three days running at a posology of 50 mg/kg (Barr *et al.*, 1994; Zajac *et al.*, 1998). Oxfendazole also has a significant effect on this flagellate protozoan in addition to its anthelmintic large potency, including *Dipylidium caninum*. Health measures are still essential. This study shows that, without boxes disinfection and washing, dogs quickly get infected again.

Giardiosis is nowadays considered by many investigators as one of the main digestive parasitosis in dogs and cats, in kennels but also at the owner's place. Some strains of this protozoan have a zoonotic aspect: some animal strains being able to infect human. There are populations adjusted to each kind of host, but interspecific transmissions are still possible (Capon, 1989; Gasser, 1990; Hopkins, 1997; Meloni *et al.*, 1995). Screening and struggle measures against this enteritic parasite are bound to expand. They will join health and medical measures. The treatment of infected dogs is one point, the vaccine development another one (Olson *et al.*, 1997; Olson *et al.*, 2000). Since a few months, a vaccine has been commercialised for dogs in kennels (Giardia Vax®, Fort Dodge Laboratories) (Olson *et al.*, 2000). The dogs show no diarrhea but the excretion of kysts is still present. Like for other parasitosis, the control measures will be integrated and based on both vaccination in collectivities, treatments, and disinfection in kennels. As the cats are often infected by *Giardia intestinalis*, the possibility of treatment with benzimidazoles should now be investigated in this species.

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