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Efficacy of zoalene and clopidol in dogs experimentally infected with Isospora canis

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EFFICACY OF ZOALENE AND CLOPIDOL IN DOGS EXPERIMENTALLY INFECTED WITH ISOSPORA CANIS

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	v
INTRODUCTION	1
REVIEW OF LITERATURE	3
ISOSPORAN SPECIES IN DOGS	3
CHEMOTHERAPY	5
Zoalene	10
Clopidol	11
COCCIDIOSIS AND TOXOPLASMOSIS	13
MATERIALS AND METHODS	16
RESULTS	18
DISCUSSION	26
SUMMARY AND CONCLUSIONS	30
LITERATURE CITED	32
APPENDIX	41

LIST OF TABLES

Table		Page
I.	Oocyst Discharge Pattern and Clinical Symptoms in 5 Dogs Experimentally Infected with 100,000 Oocysts of Isospora canis	19
II.	Oocyst Discharge Pattern and Clinical Symptoms in 13 Dogs Experimentally Infected with 100,000 Oocysts of Isospora canis and Treated with Zoalene (15, 30 and 50 mg/kg Body Weight)	20
III.	Effect of Zoalene in 4 Non-infected Dogs	23
IV.	Oocyst Discharge Pattern and Clinical Symptoms in 4 Dogs Experimentally Infected with 100,000 Oocysts of Isospora canis and Treated with Clopidol (50 mg/kg Body Weight)	24
V.	Tabulation of Daily Oocyst Discharge/gm of Feces for 5 Dogs Experimentally Infected with 100,000 Oocysts of Isospora canis	42
VI.	Tabulation of Daily Oocyst Discharge/gm of Feces for 13 Dogs Experimentally Infected with 100,000 Oocysts of Isospora Canis and Treated with Zoalene (15, 30 and 50 mg/kg Body Weight)	43
VII.	Tabulation of Daily Oocyst Discharge/gm of Feces for 4 Dogs Experimentally Infected with 100,000 Oocysts of Isospora canis and Treated with Clopidol (50 mg/kg Body Weight)	44

INTRODUCTION

Isospora canis (Protozoa:Nemeseri, 1959) is an important coccidian parasite of domestic dogs. Ingestion of the sporulated oocysts causes the release of sporozoites which invade the epithelial lining of the intestine. Schizogony and gametogony take place in the epithelial tissue resulting in a massive proliferation of the parasite and extensive destruction of the intestinal cells (Lepp and Todd, 1974). Severe infections cause loss of appetite, diarrhea and dysentery (Levine, 1973).

The recent implication of isosporan-like organisms with human toxoplasmosis (Frenkel et al., 1970; Dubey et al., 1970; Hutchison et al., 1970) suggests that members of the genus Isospora may be of significant public health importance. Although only Isospora spp. from the cat have been found to be associated with toxoplasmosis thus far (Kuhn et al., 1972), all isosporan species are morphologically similar. This suggests that information regarding one isosporan species may facilitate better control of related species.

Isospora canis in Utah has been mentioned only twice in the literature--once with respect to obtainment of oocysts (Bunch, 1969), and later for a project on oocyst sporulation rate and survival at various temperatures (Loveless, 1974). Although no surveys for I. canis have been reported in Utah, it is possible that numerous dogs in this region are exposed to and harbor the parasite at one time in their life. In six studies in the United States and Canada, 6 to

24% of the dogs examined harbored I. canis (Gassner, 1940; Levine and Ivens, 1965; Levine, 1973). This relatively high incidence is possibly due to the rapid proliferation of the parasite, and the fact that the organism is easily passed from host to host without the awareness of the owner.

Control of coccidiosis by chemotherapeutic means is common in the poultry industry, where millions of dollars are spent annually in the purchasing and manufacturing of coccidiostats and coccidiocides (Reid, 1961). Two drugs which are widely used for avian coccidiosis are Zoalene[1] (Hymas and Stevenson, 1963), trade name Zoamix, which is efficacious against the schizont stage (Reid, 1972); and Clopidol[2] (Long and Millard, 1967; Hart et al., 1967), trade name Coyden, which acts against the sporozoite stage in the life cycle (Reid, 1972).

Information on the effectiveness of known chemotherapeutic agents against coccidiosis in carnivores is limited. The objective of this project was to determine (1) the possible efficacy of Zoalene and/or Clopidol against I. canis in dogs, and (2) the practicality of using these drugs for natural infections.

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[1]Zoalene (3,5-dinitro-o-toluamide); The Dow Chemical Company, Midland, Michigan.

[2]Clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol); The Dow Chemical Company, Midland, Michigan.

REVIEW OF LITERATURE

ISOSPORAN SPECIES IN DOGS

Coccidia were first observed in 1854 by Fink (Wenyon, 1923; Gassner, 1940) in the intestinal wall of cats. In 1860, Virchow found similar organisms in dogs (Gassner, 1940). Several good review articles and/or books discussing the early work in coccidiosis have been written (Wenyon, 1923 and 1926; Becker, 1934; Gassner, 1940). Until 1959, Isospora bigemina, Isospora felis and Isospora rivolta were accepted as occurring in both dogs and cats, as well as Eimeria canis in dogs alone. Eimeria canis was first cited by Wenyon (1923). It was assumed that all 3 isosporan species were cross-transmissible, although only I. felis (Wenyon, 1923) and I. rivolta (Grassi, 1879) were described from the cat. Investigators accepted this cross-transmission theory without apparent question (Andrews, 1927; Lee, 1934; Gassner, 1940).

The theory was questioned by Nemeseri (1959) and disproven with the canine variety of I. felis. This was then renamed Isospora canis by Nemeseri (1959); Shah (1970a) confirmed Nemeseri's work. Dubey (1975) further repudiated the theory by proving that I. rivolta in cats could not be transmitted to dogs, and that I. rivolta in dogs could not be transmitted to cats. Dubey (1975) proposed the name Isospora ohioensis n. sp. for the latter species in dogs. This name will be used hereafter to designate the previous I. rivolta in dogs. Dubey did, however, prove that while neither the dog nor the

cat would pass oocysts which had originated from the opposite host, endogenous stages of I. ohioensis would develop in the cat, and endogenous stages of I. rivolta would develop in the dog. This work refuted the exception to host specificity for dog and cat coccidia as proposed by Andrews (1927).

The sporulation of the oocysts has been described for I. bigemina (Wenyon, 1923; Lee, 1934; Gassner, 1940; Levine and Ivens, 1965; Levine, 1973), for I. canis (Wenyon, 1923; Lee, 1934; Nemeseri, 1959 and 1960; Lepp and Todd, 1974; Loveless, 1974), for I. felis (Wenyon, 1923; Lee, 1934; Shah, 1970b), for I. ohioensis (Wenyon, 1923; Levine and Ivens, 1965; Mahrt, 1968; Dubey, 1975), and for I. rivolta (Wenyon, 1923; Levine and Ivens, 1965; Dubey, 1975). Under favorable environmental conditions, oocysts of I. canis sporulate within 2 to 4 days (Hall and Widgor, 1918; Nemeseri, 1959; Pellerdy, 1965). Isospora canis and I. felis differ in host and location of the parasite (Lepp and Todd, 1974), and I. rivolta and I. ohioensis apparently differ only in host (Dubey, 1975).

The endogenous stages of some of the isosporan parasites of dogs and cats have been studied in great detail. Mahrt (1967) described these stages for I. ohioensis; Lepp and Todd (1974) did the same for I. canis. Dubey and Frenkel (1972) showed that intestinal stages of I. felis and I. ohioensis in cats and dogs, respectively, could develop upon the ingestion of the extra-intestinal tissue from the opposite carnivore host. The prepatent period for such infections was either equal to or shorter than the prepatent period following ingestion of oocysts. Dogs and cats can also contract these species of Isospora after ingesting extra-

intestinal tissue from rodent intermediate hosts (Frenkel and Dubey, 1972).

Lepp and Todd (1974) showed that the endogenous stages of I. canis occur directly beneath the epithelium of the distal portion of the small intestinal villi, and that 3 asexual generations are present. First generation schizonts are found between 5 to 7 days post inoculation, whereas the second the third generation schizonts occur between 6 to 7 and 6 to 8 days, respectively. Gametes develop between days 7 to 10, and oocysts are present in the tissue between days 8 to 10. The prepatent period is 9 to 11 days, with a 10-day mean. The disease is self-limiting and appears to give strong and lasting immunity.

CHEMOTHERAPY

Chemotherapeutic as well as chemoprophylactic measures have long been used as means to either help cure or prevent both human and animal parasitic infections (Faust et al., 1975; Merck and Co., Inc., 1972 and 1973). Levine (1973) thoroughly substantiated the efficacy of numerous chemotherapeutic agents for parasitic protozoa. Pellerdy (1965) as well as Hammond (1973) have written extensively about the coccidia, and described known and proposed chemotherapeutic agents against these organisms.

In 1951, Altman reported the use of aureomycin, atabrine and azamine against clinical cases of canine coccidiosis. He found that while both atabrine and azamine were effective in eliminating coccidian oocysts, they had to be carefully administered because intestinal irritation frequently occurred. Aureomycin was reported

to be efficacious and with no apparent intestinal irritation and no toxic effects, regardless of dosage administered.

Perry (1952) reported the use of coccithane in treating dogs with clinical coccidiosis, and reviewed the literature pertinent to incidence and symptomology of the disease. Although all 3 isosporan species of canine coccidia, as well as Eimeria canis were identified in his work, no species specific trials were conducted. Perry had noted unfavorable results in previous attempts using sulfamezathine and sulfaguanidine, with 90 to 95% mortality in the treated dogs with secondary infections. In 56 dogs treated with coccithane (2 gr/lb body wt/day, divided into 3 equal doses) no toxic effects were seen and mortality was less than 10%. Perry also noted that clinical canine coccidiosis is not a self-limiting disease in many instances. Frequently the infection lowers resistance, allowing secondary organisms to become active, thereby complicating and prolonging the course of the disease. Death can result where treatment is inadequate.

Fernando (1956) reported the use of both sulfamezathine and sulfaguanidine for treatment of clinical coccidiosis caused by I. ohioensis and I. bigemina in 14 dogs in Ceylon. Twelve dogs received sulfamezathine orally (1 gm/15 lb body weight), and 2 dogs received sulfaguanidine at the same dosage. The dogs also received two thirds the original dosage daily for the next 3 days. No data were given in the results other than the conclusion that sulfamezathine and sulfaguanidine appear to be efficacious in canine coccidiosis.

Fisher (1958) recommended the use of nitrofurazone in the treatment of complications arising from canine coccidiosis, although dosage levels and coccidial species involved were not stated. Smith (1959) and Smith and Edmonds (1959) concurred with Fisher's recommendation concerning nitrofurazone. These workers used 18 dogs, all exhibiting clinical coccidiosis; however, no parasite speciation was given. All dogs were treated with nitrofurazone at 2 mg/lb body weight, thrice daily for 10 days. An additional group of 7 dogs with clinical coccidiosis were left untreated, except for an improved diet fortified with vitamins. The feces of 2 of the treated animals were negative for oocysts after 11 and 31 days post treatment, whereas 4 of the untreated animals passed oocysts for 3 months; 2 of the untreated dogs were oocyst free within a month.

Duberman (1960) reported the results of a study comparing nitrofurazone and 3 combined sulfonamides (sulfamezathine, sulfathiazole and sulfamerazine), for treatment of I. canis in 40 dogs obtained from various animal shelters. All of the dogs had clinical coccidiosis prior to treatment. Half of the dogs received nitrofurazone orally in 3 equal daily doses with a varied total dosage of 4 to 10 mg/lb body weight. The other 20 dogs were treated with the combined sulfonamides (1 gr/lb body weight) divided into 3 daily doses. Treatment lasted 5 to 20 days, or until 5 consecutive negative fecal examinations were obtained. In the 20 dogs treated with nitrofurazone, the time from the first treatment to the first negative fecal examination ranged from 3 to 25 days with an average of 9.3 days. In the sulfonamide group the patent period was 4 to 23

days with an average of 9.7 days. Both drugs were reported as being effective against I. canis; however, no control animals were used.

Rachman and Pollock (1961) used nitrofurazone and sulfaguanidine to treat 15 dogs with clinical coccidiosis. The dogs were vaccinated against distemper and hepatitis and were divided into 3 groups; 5 were treated with nitrofurazone (2 mg/lb body weight, given twice daily), 5 were treated with sulfaguanidine (1 gr/lb body weight, given daily), and 5 served as untreated controls. The dogs in the medicated groups were treated for one week, at which time all animals in the nitrofurazone group were all negative for oocysts. In the dogs treated with sulfaguanidine, 4 of the 5 were still passing oocysts as were 3 of the 5 untreated controls. The sulfaguanidine treated dogs were therefore given an additional week of treatment, at which time 3 of the 5 dogs were still passing oocysts, as were 3 of the 5 untreated controls. The sulfaguanidine group was then treated with nitrofurazone (2 mg/lb body weight, given twice daily) for one week. After the third week of treatment all 5 treated dogs were negative for coccidia; however, no comparative results were mentioned for the untreated controls. Rachman and Pollock concluded that 2 mg of nitrofurazone/lb body weight, given twice daily effectively controlled canine coccidiosis, whereas the results with sulfaguanidine were similar to those from untreated controls.

Knight (1962) reported on the increased pathogenicity of canine coccidiosis when dogs were concurrently infected with distemper, respiratory and/or gastrointestinal bacterial infections.

Each of these infections tended to increase the virulence of the other, especially the interaction of coccidiosis and distemper. Knight suggested that uncomplicated coccidiosis is rarely fatal and responds to a variety of anticoccidial agents, but that complicated coccidiosis is more difficult to treat. Knight's initial attempts using doses of phthalysulfathiazole and chlorotetracycline to provide systemic antibacterial action were not effective. He further reported on the combined usage of sulfadimethoxine and hyperimmune canine globulin concentrate (Globulon) in treating 25 dogs with complicated coccidiosis. Eighteen of the 25 dogs recovered, with prompt disappearance both of oocysts and clinical symptoms. Five of the dogs had the clinical symptoms controlled while continuing to shed oocysts, and 2 of the dogs died of encephalitis. Knight concluded that a parenteral administration of concentrated canine globulins coupled with a large dose of sulfadimethoxine was highly effective in the treatment of canine coccidiosis.

Whitney (1962) questioned whether or not canine coccidiosis could be cured. From his work with sulfaguanidine, he concluded that the drug was practically useless. With reference to work by others where excellent success with sulfaguanidine and other anti-coccidials were reported, Whitney noted that these researchers failed to use controls or make daily fecal counts. He further concluded that all veterinarians can do is alleviate the symptoms, and that coccidiosis is "as natural an occurrence in maturing as teething."

Smart (1971) reported the use of amprolium in treating canine coccidiosis in "several thousand puppies." He switched to

amprolium because of its known efficacy against coccidiosis in poultry and because of unsatisfactory results with other anti-coccidial drugs he had tried. His recommended dosage level was 100 mg/pup daily for 7 days. No data were given as to exact numbers of dogs treated, coccidial species involved, whether or not controls were used, or other information which would confirm his statements of purported efficacy.

Zoalene

Zoalene (Zoamix) was first placed on the commercial market in 1960 (Reid, 1961 and 1972). Several investigators have attested to its efficacy against various species of Eimeria in chickens: E. acervulina and E. burnetti (Hymas and Stevenson, 1963; Reid et al., 1969), E. maxima (Hymas and Stevenson, 1963), E. mivati (Reid et al., 1969), E. tenella (Blount and Scott, 1960; Hymas and Stevenson, 1960 and 1963; Joyner, 1960; Kirsch, 1962; Marthedal and Velling, 1963; Reid et al., 1969), and E. necatrix (Blount and Scott, 1960; Hymas and Stevenson, 1960 and 1963; Joyner, 1960; Reid et al., 1969).

The compound is a benzamide which has its greatest activity against the developing first-generation merozoites (Reid, 1972). Depending on the species of Eimeria involved, Zoalene can be either coccidiostatic or both coccidiostatic and coccidiocidal (Reid et al., 1969). As new coccidiostats are developed, Zoalene has become one of the standard reference drugs to which the new coccidiostats are compared (Long and Millard, 1967; Reid and Brewer, 1967).

In 1963, Hymas and Stevenson found Zoalene to be efficacious against Eimeria meleagrimitis, E. adenoides and E. gallopavonis in turkeys. It has also been reported to be efficacious against coccidiosis in lambs and calves (Sanger et al., 1961; Eckman and Casorso, 1972). However, in 1963, Peardon et al. stated that Zoalene as well as three other poultry coccidiostats (glycarbylamide, nitrofurazone and framycetin sulfate) and 2 routinely used bovine coccidiostats (sulfaguanidine and sulfamethazine) were ineffective against natural bovine coccidial infections.

In unpublished studies on the toxicity of Zoalene, it was found that dogs on dosage levels of approximately 10, 5 and 2.5 mg/kg body weight/day showed no adverse effects (Hymas, 1960).

The aforementioned uses for Clopidol and Zoalene attest to the varied and increasing therapeutic scope of the poultry coccidiostats.

Clopidol

Clopidol (Coyden), also known as meticlorpindol or clopindol, was first introduced to the commercial market in 1968 (Reid, 1972), although experimental data concerning it can be found in the literature as early as 1967 (Reid and Brewer, 1967; Stock et al., 1967). The compound is a pyridinol (3,5-dichloro-2,6-dimethyl-4-pyridinol) (Ryley, 1967; Reid, 1972), which is highly effective against numerous species of Eimeria in poultry, namely: Eimeria acervulina, E. burnetti and E. miyati (Reid and Brewer, 1967; Hymas, 1967; Long and Millard, 1967; Stock et al., 1967; Reid et al., 1969), E. maxima (Reid and Brewer, 1967; Hymas, 1967; Long and Millard,

1967; Stock et al., 1967), E. mitis (Hymas, 1967; Stock et al., 1967), E. necatrix (Reid and Brewer, 1967; Hymas, 1967; Long and Millard, 1967; Stock et al., 1967), and E. tenella (Reid and Brewer, 1967; Hymas, 1967; Long and Millard, 1967; Stock et al., 1967; Reid et al., 1969). The recommended dosage level is 0.0125% of the diet or approximately 0.25 lb/ton of feed. Reid and Brewer (1967) found that Clopidol not only inhibited the morbidity and mortality rates in poultry, but also increased wight gain and feed conversion efficacy.

Clopidol acts against the sporozoite stage of the parasite once it has invaded the intestinal mucosa (Hymas, 1967; Long and Millard, 1968; Reid, 1972). If it is not present on the first day of infection, its efficacy is almost negligible (Ryley, 1967; Reid, 1972). Because of its efficacy in the early part of the life cycle, the parasite does not become antigenic to the host, therefore no immune response is elucidated (Long and Millard, 1968).

Although Clopidol is primarily a coccidiostat (Reid and Brewer, 1967; Stock et al., 1967; Reid, 1972), if treatment is continued more than 77 days, it is coccidiocidal as well (Long and Millard, 1968; Reid et al., 1969). If discontinued earlier than the 77 day period, latent coccidiosis may occur.

Clopidol has also been shown to be efficacious against other parasitic agents. Markley et al. (1972) reported its activity against numerous species of malaria: Plasmodium berghei in mice, P. gallinaceum in chicks, P. cynomologi in the macaca mulatta monkey, and the refractory (chloroquine resistant) strain of P. falciparium in humans. Chroust (1973) reported beneficial effects using

Clopidol against numerous natural infections in lambs, namely: Eimeria arloingi, E. crandallis, E. faurei, E. intricata, E. ninakohlyakomovae and E. parva. It was later shown to be effective against Leucocytozoon smithi in turkeys (Siccardi et al., 1974).

Dosages up to 200 mg/kg/day have been shown to have no toxic effects when administered to dogs for a period of 2 years (McCollister, Brown, and Sadek, 1966; Stockhouse, 1966).

COCCIDIOSIS AND TOXOPLASMOSIS

Prior to the past decade, the tie between coccidiosis and toxoplasmosis was limited to the fact that the causative organisms of both were considered sporozoans (Jacobs, 1967, 1973 and 1974); however, no additional relationships were known to exist. In 1965, Hutchison proposed the idea that Toxoplasma gondii might be passed in the ova of the nematode, Toxocara cati, a proposal which led to an increased worldwide research effort on toxoplasmosis (Frenkel, 1973a; Jacobs, 1973).

Since that time voluminous material has been written concerning toxoplasmosis regarding its taxonomy and basic morphology, a new proposed life cycle, and information on its inter-relationship with the coccidia (Frenkel, 1973b; Levine, 1973). Jacobs (1973) obtained over 2,000 citations dealing with toxoplasmosis from the National Library of Medicine from the years 1967 to 1972. Several good review articles and/or books are available concerning the present knowledge of toxoplasmosis: Jacobs, 1967, 1973 and 1974; Levine, 1973; Feldman, 1974; Frenkel, 1973a.

Following Hutchinson's original hypothesis, it was proven that Toxocara cati was not involved in the Toxoplasma life cycle, but rather that T. gondii was being passed as a coccidian-like oocyst, probably of the genus Isospora (Frenkel et al., 1970; Hutchison et al., 1970). Parasitized cats would shed the small T. gondii oocysts (10 to 12u; Dubey, 1973) after having eaten mice previously infected with toxoplasmosis or isosporan oocysts passed from an infected cat (Frenkel et al., 1970; Frenkel, 1973a).

It was felt that T. gondii was unique in that it was the only "coccidia" which produced extra-intestinal stages and could infect an animal other than the definitive host (Frenkel, 1973a). However, recent articles dealing with Isospora felis and I. rivolta in cats, have shown that these two parasites are also capable of infecting extra-intestinal tissues of the normal definitive hosts (Dubey and Frenkel, 1972), as well as infecting extra-intestinal tissues of hosts such as mice, rats and hamsters (Frenkel and Dubey, 1972). Dubey (1975) also showed that dogs can harbor extra-intestinal stages of I. rivolta from cats, and cats can harbor extra-intestinal stages of I. ohioensis from the dog. This apparent lack of host specificity as well as the marked morphological similarity between the endogenous stages of Isospora and Toxoplasma emphasize the probable relationship of these organisms.

In 1953, Eyles reported that the only drugs then available for controlling toxoplasmosis were the sulfonamides, notably sulfadiazine, sulfamethazine and sulfamerazine. Summers (1953) also reported some success with pyrimethamine; however, Eyles noted that the sulfonamides left "something to be desired." Jacobs (1967)

concurred with Eyles, and stated that pyrimethamine's disadvantage of teratogenesis must be carefully weighed when considering its use. In 1973, Jacobs summarized work by others using spiramycin, acetylspiramycin, clindamycin and N-dimethyl-4-pentyl clindamycin, and noted that the latter two drugs were also teratogenic. Bedrnik (1972a, 1972b) reported the use of 5 coccidiostatic drugs in poultry (buquinolate, clopidol, VUFB 6207, nicarbazin and robenziden) in treating T. gondii. These drugs were chosen because of the demonstrated similarity between coccidiosis and toxoplasmosis, and the wide range of drug efficacy against Eimeria. Three of the 5 drugs were ineffective against T. gondii in cell culture; however, robenziden and nicarbazin suppressed multiplication of the parasite. When Bedrnik later used these same drugs against the parasite in infected mice, the three previously ineffective compounds, as well as nicarbazin were not effective. However, T. gondii infected mice recovered after a 7-day treatment with robenziden at 1 mg/mouse/day.

In view of this and other information, the confusion and uncertainty of the entire coccidiosis-toxoplasmosis spectra becomes more apparent, along with the need for continued research and clarification.

MATERIALS AND METHODS

Twenty six dogs of a variety of breeds, weights, and less than a year in age were obtained from the Provo and Orem City animal control shelters. The dogs were transferred to the Parasitology Animal Research Laboratory at Brigham Young University, Provo, Utah.

Upon entering, all animals were housed in 0.9 x 2.5 m kennels, which had been thoroughly cleansed prior to their arrival, in an effort to minimize natural coccidial infections. Each dog was weighed and vaccinated against distemper. Fresh fecal samples were taken for microscopic examination to determine the presence of intestinal parasites. All dogs were demonstrated to be free of observable coccidial infections prior to use in the experiment. The results from any dog which acquired a natural infection of coccidiosis during the study were not used in the evaluation of drug efficacy.

Twenty-two dogs were randomly divided into 5 experimental groups: Untreated controls, Zoalene treatment at 15, 30 and 50 mg/kg body weight, and Clopidol 50 mg/kg body weight. All treated dogs received the drug in their food on the day prior to infection with *I. canis*. The drug was placed in the dog's feed throughout the treatment period with the exception of dogs nos. 13, 32, 33, 34, 35 which received the drug orally in capsule form. On the day following treatment all animals were infected per os with 100,000 *I. canis* oocysts. Four additional dogs (nos. 30 to 33)

served as non-infected, treated controls for one trial on assessment of drug toxicity of Zoalene. The original source oocysts came from dogs which were supplied through Merck and Co., Rahway, New Jersey.

Treatment was stopped on day 15 following infection or when observable toxic effects first occurred. Daily fecal samples were taken throughout the prepatent and patent periods.

The kennels were washed daily and all fecal matter removed. Observations as to clinical appearance of the dog and any pathogenic or toxic effects caused by either the coccidia or the chemotherapeutic agent were noted and recorded. Following the patent period all animals were killed with an intracardial injection of sodium pentabarbitol.

All fecal samples were diluted in 50% Sheather's sugar solution, after which aliquot portions were placed in a McMaster's Counting Chamber. The material was then microscopically examined with the aid of a compound microscope. Counts of oocysts per gram of feces were determined and recorded. (See appendix: Tables V, VI, and VII.) The efficacy of the 2 drugs tested was assessed by comparing the data for the oocyst discharge pattern and observable clinical symptoms noted by use of the student's "t" test. Differences between sample means were considered significant when $P < 0.05$.

Oocysts from the untreated controls were collected on days of peak discharge and stored for use in subsequent groups of animals. The collection method and storage technique for the oocysts was the same as described by Loveless (1974).

RESULTS

Table I indicates the oocyst discharge pattern and clinical symptoms for 5 untreated dogs experimentally infected with 100,000 oocysts of Isospora canis. These animals showed a mean onset of patency of 9 days with a mean for the day of peak oocyst discharge of 12.2 days and an oocyst discharge duration of 8.2 days. The average peak oocyst count for these 5 dogs was 310,820 oocysts per gram of feces (o.p.g.) with a mean total count (total summation of the daily counts) of 759,110 o.p.g. All dogs in the control group showed at least 1 day of diarrhea (mean = 6.6 days) and 2 dogs showed at least 1 day of bloody feces. No paralysis was noted in any of the non-treated dogs.

Table II indicates the results of 13 dogs experimentally infected with 100,000 oocysts of I. canis and treated with Zoalene at 15, 30 or 50 mg/kg body weight. For 6 dogs treated at 15 mg/kg body weight the mean onset for patency was 12.2 days, the mean day of peak oocyst discharge was 15.3 days and mean length in days during which oocysts were discharged was 7.2 days. For the 3 dogs treated at 30 mg/kg, these figures were 11, 15.3 and 8.7 days, respectively; and for the 4 dogs treated at 50 mg/kg the values were 12.2, 16.5 and 7.5 days, respectively. All of the values for the mean onset of patency and that of the mean day of peak oocyst discharge were statistically significant from the non-treated controls, whereas the mean number of days oocysts were

Table I. Oocyst discharge pattern and clinical symptoms in 5 dogs experimentally infected with 100,000 oocysts of Isospora canis.

Dog no.	Oocysts given	Dosage (mg/kg)	Oocyst discharge pattern					Symptoms		
			Day patent	Peak count (o.p.g.)	Day of peak	Total count* (o.p.g.)	Duration (days)	Diarrhea (days)	Bloody feces (days)	Paralysis (days)
18	100,000	none	9	117,000	12	249,100	9	1	0	0
19	100,000	none	9	258,500	12	614,500	7	3	0	0
20	100,000	none	9	548,600	12	1,311,300	8	4	0	0
38[a]	100,000	none	9	135,000	13	386,250	6	12	2	0
39	100,000	none	9	495,000	12	1,334,400	11	13	1	0
Means:	100,000	none	9	310,820	12.2	759,110	8.2	6.6	.6	0

[a]Died on day 14.

[b]Total summation of the daily counts.

Table II. Oocyst discharge pattern and clinical symptoms in 13 dogs experimentally infected with 100,000 oocysts of Isospora canis and treated with zoalene (15, 30 and 50 mg/kg body weight).

Dog no.	Protocol			Oocyst discharge pattern					Symptoms		
	Oocysts given	Dosage (mg/kg)	Day drug withdrawn	Day patent	Peak count (o.p.g.)	Day of peak	Total count (o.p.g.)	Duration (days)	Diarrhea (days)	Bloody feces (days)	Paralysis (days)
16[a]	100,000	15	15	13	550	13	750	3	1	0	0
17	100,000	15	15	11	256,000	14	333,600	5	0	0	0
27	100,000	15	13	15	1,550	17	5,150	7	0	0	0
28	100,000	15	13	13	8,900	15	21,370	8	3	1	0
29	100,000	15	13	12	198,500	17	313,450	9	2	0	0
35	100,000	15	12	9	340,000	16	1,161,250	11	4	0	0
Means:	100,000	15	13.5	12.	134,250	15.3	305,928	7.2	1.7	.2	0
Statistical significance				.005	N.S.	.005	N.S.	N.S.	.05	N.S.	N.S.
24	100,000	30	13	11	274,000	16	811,250	14	5	1	1
25	100,000	30	13	11	3,550	15	6,470	7	1	0	0
26[b]	100,000	30	13	11	71,700	15	140,600	5	7	7	0
Means:	100,000	30	13	11	116,416	15.3	319,4 0	8.7	4.3	2.7	.3
Statistical significance				.005	N.S.	.0005	N.S.	N.S.	N.S.	N.S.	N.S.

Table II. (Continued)

Dog no.	Protocol			Oocyst discharge pattern					Symptoms		
	Oocysts given	Dosage (mg/kg)	Day drug withdrawn	Day patent	Peak count (o.p.g.)	Day of peak	Total count (o.p.g.)	Duration (days)	Diarrhea (days)	Bloody feces (days)	Paralysis (days)
14	100,000	50	12	12	500	14	1,300	4	0	0	3
21	100,000	50	13	12	750	15	2,065	5	1	0	4
22	100,000	50	13	14	3,400	18	10,200	10	4	0	0
23	100,000	50	10	11	8,650	19	31,650	11	3	0	6
Means:	100,000	50	12	12.2	3,325	16.5	11,304	7.5	2	0	3.3
Statistical significance				.0005	.01	.005	.025	N.S.	.05	N.S.	.025[c]

[a]Appeared weak on day 15; died day 16.

[b]Extremely weak on day 15 from blood loss; died day 16 (intussusception).

[c]Value significantly higher than that of non-treated controls.

discharged did not differ significantly. The peak oocyst discharge and total oocyst count in the 15 and 30 mg/kg treated dogs did not differ significantly from the untreated controls, however the values in the 50 mg/kg treated dogs were significantly different. With respect to a statistical comparison of clinical symptoms observed, the mean number of days of diarrhea in the dogs treated with either 15 mg/kg or 50 mg/kg, as well as the number of days of paralysis in the latter group differed significantly from that of the non-treated controls. Zoalene at 50 mg/kg body weight had the most marked results for any individual group, and showed significantly different results from the untreated controls in all categories except duration of patency and days of bloody feces. However, it also produced the greatest toxicity in the dogs. Three of the 4 dogs infected with I. canis and treated with Zoalene at 50 mg/kg, as well as both of the treated, non-infected dogs (nos. 32 and 33; Table III) showed paralytic symptoms due to the toxic effects of the drug. No paralysis was noted in any of the other dogs in this study. Zoalene, therefore, showed definite efficacy against I. canis, but could not be considered as a practical compound because of the associated toxicity it produces at efficacious levels.

Table IV shows the effect of Clopidol at 50 mg/kg body weight on 4 dogs experimentally infected with 100,000 oocysts of I. canis. The mean onset for patency in this group was 10.5 days (significant at .025 level), with an average day of peak oocyst count of 15.3 days (significant at .005 level), and an average duration of oocyst discharge of 9.5 days (non-significant). The mean peak

Table III. Effect of zoalene in 4 non-infected dogs.

Dog No.	Protocol		Weight pattern (lbs.)			Symptoms	
	Dosage (mg/kg)	Day terminated	Day 0	Day 15	Weight gain or loss	Spasma (days)	Paralysis (days)
30[a]	15	14	12.5	12.5	0	0	0
31[a]	15	14	13	13	+ .5	0	0
Means:	15	14	12.75	13	+ .25	0	0
32[b]	50	3	13	10[c]	-3	1	2
33[b]	50	2	7	6[c]	-1	2	3
Means:	50	2.5	10	8	-2	1.5	2.5

[a]Contracted natural infections of I. canis.

[b]Weight at time of death.

[c]Died on day 4.

Table IV. Oocyst discharge pattern and clinical symptoms in 4 dogs experimentally infected with 100,000 oocysts of Isospora canis and treated with Clopidol (50 mg/kg body weight).

Dog no.	Protocol			Oocyst discharge pattern					Symptoms		
	Oocysts given	Dosage (mg/kg)	Day drug withdrawn	Day patent	Peak count (o.p.g.)	Day of peak	Total count (o.p.g.)	Duration (days)	Diarrhea (days)	Bloody feces (days)	Paralysis (days)
5	100,000	50	15	11	50,800	16	112,850	12	6	1	0
6	100,000	50	15	12	1,000	13	2,500	4	6	0	0
10	100,000	60	15	9	91,200	15	249,100	12	4	0	0
11	100,000	50	15	10	24,500	17	48,250	10	4	0	0
Means:	100,000	50	15	10.5	41,875	15.3	103,175	9.5	5	.25	0
Statistical significance:				.025	.025	.005	.025	N.S.	N.S.	N.S.	N.S.

oocyst count was 41,875 o.p.g. (significant at .025 level), and the total oocyst discharge count was 103,175 o.p.g. (significant at .025 level). Data on the clinical symptoms noted in these dogs treated with Clopidol were not significantly different from the non-treated control dogs. Clopidol, therefore, gave moderate coccidiostatic effect, but not as marked as that noted with Zoalene. In addition, there were no noticeable toxic effects noted in any of the dogs which received this compound.

DISCUSSION

The assessment of coccidiostatic efficacy of Zoalene and Clopidol was based on the ability of the chemotherapeutic agent to inhibit or alter the Isospora canis infection. Such changes were demonstrated in the current study by a prolonged mean prepatent period and day of peak oocyst discharge, a decreased mean peak oocyst and mean total oocyst discharge, and a reduction in the number of days the animals had clinical symptoms. Reid et al. (1969) noted that for chemotherapeutic trials in coccidiosis, daily oocyst counts must be made throughout the prepatent and patent periods in order to determine if latent coccidiosis would occur following drug withdrawl. They further noted that if only a single parameter were to be used in demonstrating latent coccidiosis, oocyst counts would be the most useful. An evaluation of clinical symptoms in any parasitic disease such as coccidiosis remains difficult to objectively quantitate.

In the present study, Zoalene was shown to have a marked coccidiostatic effect against I. canis and reduced the mean peak and total oocyst counts by 99% as compared with infected, non-treated controls. However, it was noted that at 50 mg/kg the animals experienced toxic effects from the drug. These effects were first observed as uncoordinated movements when the animal walked, but eventually progressed to paralysis of the hind quarters followed by paralysis of the fore legs. Withdrawal from treatment led to a return

of normal locomotive patterns within 3 to 4 days in 4 of the 6 afflicted animals. However, 2 non-infected, treated dogs receiving the agent in capsule form for 2 and 3 days, respectively, died soon after medication was removed. This high toxicity at therapeutic levels precludes Zoalene from serious consideration as a coccidiostatic agent for I. canis. This is the first reported attempt to use Zoalene as a treatment for experimental infections of Isospora in any host.

Clopidol was shown to be moderately coccidiostatic against I. canis and reduced the mean peak and total oocyst counts by 86%, as compared with non-treated controls. Clopidol, however, did not produce the toxic effects observed in the use of Zoalene. This is also the first reported use of Clopidol against Isospora in any host.

When the current work is compared to previously published studies, several differences become apparent. First, most of the previous research on potential coccidiostats has been conducted with bacteriostatic agents: aureomycin (Altman, 1951), coccithane (Perry, 1952), sulfonamides (Fernando, 1956; Duberman, 1960; Rachman and Pollock, 1961), nitrofurazone (Fisher, 1958; Smith, 1959; Smith and Edmonds, 1959; Duberman, 1960; Rachman and Pollock, 1961), tetracyclines and canine antibodies (Knight, 1962). Other than the work done by Smart (1971) with amprolium, this is the only reported trial where a drug developed primarily as a coccidiostat has been used in an attempt to treat canine coccidiosis.

Secondly, it is the only time when coccidia-free dogs have been selected for a trial, the animals subsequently infected with a

uniform innocula, and the disease monitored with daily fecal samples throughout the prepatent and patent periods. All previous investigators have used animals exhibiting clinical coccidiosis prior to treatment with a chemotherapeutic agent. Inasmuch as most coccidiostats are efficacious during the early stages of the prepatent period (Levine, 1963; Reid, 1972), they must be administered at time of exposure or as soon thereafter as possible, in order to be efficacious (Levine, 1963). Anticoccidial treatment initiated after symptoms appear provides little protection (Reid, 1972). Therefore, the administration of these other purported coccidiostats during the patent period would have little, if any, effect. However, inasmuch as they are primarily bacteriocidal, they may help reduce secondary bacterial infections in the coccidia-traumatized cells (Whitney, 1962).

Thirdly, other than limited control methods used by Rachman and Pollock (1961), this is the only reported study where a carefully controlled protocol has been used to verify whether or not the chemotherapeutic agent was actually efficacious. Levine (1963) noted that inasmuch as coccidiosis is a self-limiting disease, non-efficacious agents have sometimes received purported efficacy, since the disease subsided after they were administered. Whitney (1962) stated that some reported coccidiostats were practically useless, because the initial work on the drug was done without comparison to infected, non-treated control animals, and daily fecal counts were not determined. Since the host can recover spontaneously, chemotherapeutic trials need to be carefully controlled in order to accurately assess the efficacy of the agent.

Fourthly, all dogs selected were less than a year in age, which minimized the possibility of obtaining previously infected dogs with natural immunity. In addition, all dogs were vaccinated against distemper to eliminate the possible increased pathogenicity from the coccidiosis-distemper interaction noted by Knight (1962).

Although this study did not result in the identification of practical canine coccidiostats, it is probable, however, that successful compounds will be identified in the near future. It does show need for further research in the area of attempted chemotherapy, possibly by using agents which have proven or will be proven to be efficacious against toxoplasmosis. As additional work is completed on either the life cycle of chemotherapy of Isospora and Toxoplasma, further understanding of the currently complex relationship of these genera will likely be elucidated.

SUMMARY AND CONCLUSIONS

The effects of Zoalene and Clopidol on coccidia-free dogs experimentally infected with 100,000 oocysts of Isospora canis were determined. From this study the following conclusions can be drawn:

1. Zoalene, when tested at levels of 15, 30, or 50 mg/kg body weight, was coccidiostatic against I. canis in dogs. It significantly prolonged the mean prepatent period and the mean day of peak oocyst discharge at all dosage levels tested, as compared to the non-treated controls. In addition, at the 50 mg/kg level, it significantly decreased the mean peak oocyst count, the mean total oocyst count, and the mean number of days of diarrhea observed. However, 5 of the 6 animals treated at that level showed paralytic symptoms (Tables II and III), and 2 non-infected, treated animals within that group died on day 4 after having received the drug for an average of 2.5 days (Table III). Because of these toxic effects at the therapeutic level, Zoalene cannot be considered a practical compound for the treatment of canine coccidiosis.

2. Clopidol, when used at 50 mg/kg body weight, was moderately coccidiostatic against I. canis, but did not show the marked efficacy noted with the comparable level of Zoalene. Dogs treated with Clopidol showed an 86% reduction in the mean peak and total oocyst counts, whereas dogs treated with Zoalene at that upper level showed a 99% reduction in those values. Nevertheless, treatment with Clopidol significantly prolonged the mean prepatent period

and the mean day of peak oocyst discharge, and significantly decreased the mean peak oocyst and total oocyst counts as compared to the non-treated controls. However, there were no significant differences noted in days duration of oocyst discharge or in observable clinical symptoms (Table IV). In contrast with Zoalene, treated dogs showed no noticeable toxic effects from the drug. Therefore, although Clopidol gave limited coccidiostatic effects, it would probably not be considered a practical drug for treatment of I. canis in dogs.

3. A standard protocol should be developed to include mandatory guidelines for evaluating chemotherapeutic agents in any self-limiting parasitic disease such as canine coccidiosis.

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APPENDIX

Table V. Tabulation of daily oocyst discharge/gm of feces for 5 dogs experimentally infected with 100,000 oocysts of Isospora canis.

Dog no.	Day																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
18	0	0	0	0	0	0	0	0	200	15,750	44,100	117,000	59,900	10,000	3,600	350	100	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	500	53,300	97,900	258,500	101,000	60,000	43,300	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	2,000	101,250	372,000	548,600	200,000	75,100	11,500	850	0	0	0	0	0	0	0	0	0
38[a]	0	0	0	0	0	0	0	0	650	3,300	9,900	37,800	135,000	99,600											
39	0	0	0	0	0	0	0	0	16,000	100,000	93,000	495,000	177,500	108,500	173,400	37,800	104,500	24,800	3,900	0	0	0	0	0	0
Means:	0	0	0	0	0	0	0	0	3,870	54,720	123,380	291,380	134,300	70,640	57,950	9,750	26,150	6,200	975	0	0	0	0	0	0

[a]Died on day 14.

Table VI. Tabulation of daily oocyst discharge/gm of feces for 13 dogs experimentally with 100,000 oocysts of Isospora canis and treated with Zoalene (15, 30 and 50 mg/kg body weight).

Dog No.	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Zoalene (15 mg/kg)																									
16[a]	0	0	0	0	0	0	0	0	0	0	0	0	550	100	100										
17	0	0	0	0	0	0	0	0	0	0	N.S.	59,200	15,250	256,000	400	0	250	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	1,050	1,550	550	650	100	0	0	1,150	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	172	3,100	8,900	6,150	2,150	500	350	50	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	150	0	0	0	50	198,500	40,700	25,600	18,900	16,500	9,700	3,350	0	0
35	0	0	0	0	0	0	0	0	50	0	6,500	130,200	290,000	49,300	198,000	340,000	137,500	8,200	1,500	0	0	0	0	0	0
Means:	0	0	0	0	0	0	0	0	8	0	1,300	31,592	50,995	51,417	34,583	69,450	67,990	9,990	5,620	3,810	3,300	1,940	900	0	0
Zoalene (30 mg/kg)																									
24	0	0	0	0	0	0	0	0	0	0	1,750	4,100	25,000	50,600	76,200	274,000	190,000	145,800	27,700	5,800	4,000	2,500	3,700	100	0
25	0	0	0	0	0	0	0	0	0	0	50	0	270	2,000	3,550	200	50	350	0	0	0	0	0	0	0
26[b]	0	0	0	0	0	0	0	0	0	0	4,500	0	10,250	50,100	71,700	4,000									
Means:	0	0	0	0	0	0	0	0	0	0	2,117	1,367	11,840	34,233	50,483	92,733	95,025	73,075	13,850	2,900	2,000	1,250	1,850	50	0
Zoalene (50 mg/kg)																									
14	0	0	0	0	0	0	0	0	0	0	0	150	400	500	250	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	100	215	400	750	0	0	600	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	500	2,750	0	250	3,400	1,000	200	400	950	600	50	0
23	0	0	0	0	0	0	0	0	0	0	50	0	800	1,500	4,650	3,750	2,400	4,850	8,650	4,150	700	150	0	0	0
Means:	0	0	0	0	0	0	0	0	0	0	0	175	354	725	2,100	9,375	662	2,212	2,412	1,087	300	275	150	12	0

[a]Appeared weak on day 15; died day 16.

[b]Extremely weak on day 15 from blood loss. Died day 16 (intussusception).

[NS]No sample obtained.

Table VII. Tabulation of daily oocyst discharge/gm of feces for 4 dogs experimentally infected with 100,000 oocysts of Isospora canis and treated with Clopidol (50 mg/kg body weight).

Dog no.	Day																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Clopidol (50 mg/kg)																									
5	0	0	0	0	0	0	0	0	0	0	400	0	0	300	500	50,800	12,350	19,700	2,800	14,750	4,950	5,000	1,250	50	0
6	0	0	0	0	0	0	0	0	0	0	0	150	1,000	650	700	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	50	1,250	4,950	3,750	12,550	56,300	91,200	29,600	24,500	17,200	7,000	750	0	0	0	0	0
11	0	0	0		0	0	0	0	0	50	100	0	300	1,200	6,900	6,500	24,500	5,900	2,700	100	0	0	0	0	0
Means:	0	0	0	0	0	0	0	0	12	325	1,362	975	3,462	14,612	24,825	21,725	15,338	10,700	3,125	3,900	1,237	1,250	312	12	0

VITA

JAMES CARSON BROWN

EFFICACY OF ZOALENE AND CLOPIDOL IN DOGS EXPERIMENTALLY INFECTED WITH *ISOSPORA CANIS*

James C. Brown

Department of Zoology

M.S. Degree, August 1976

ABSTRACT

Twenty-six coccidia-free pound dogs were each inoculated per os with 100,000 oocysts of *Isospora canis* to evaluate drugs for efficacy against coccidiosis in dogs. Zoalene was tested at levels of 15, 30, and 50 mg/kg of body weight, and Clopidol was tested at 50 mg/kg only. Efficacy was determined by comparing results obtained on the daily oocyst discharge pattern and on clinical symptoms noted in treated animals with data from infected, non-treated controls.

Zoalene produced a delay in the onset of patency and day of peak oocyst discharge, and a decrease in the days of diarrhea noted. At 50 mg/kg, a 99% reduction in mean numbers of discharged oocysts was noted. That therapeutic level, however, was markedly pathogenic to the dogs and produced paralysis and death in some cases. Clopidol produced a delay in the onset of patency and day of peak oocyst discharge, a limited (86%) reduction in mean numbers of discharged oocysts, but no significant differences in clinical symptoms observed.

Thus, neither drug to be an ideal compound for canine coccidiosis.

COMMITTEE APPROVAL: