THE EFFICACY OF RUELENE AS AN ANTHELMINTIC IN BEEF CATTLE

by

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INTRODUCTION

Because of the economic importance of the beef cattle industry in the United States and throughout the world, the losses incurred among cattle by gastrointestinal parasites are important. There is a need for practical and efficient ways to combat internal parasites. Therefore, the development of safe and effective anthelmintics is necessary to supplement management practices. However, before any parasiticide is placed on the market or made available to veterinarians, it must be thoroughly tested. All aspects of the compound must be investigated, including toxicity of the chemical and its metabolites, elimination from host and residue, and of course, the anthelmintic activity at prophylactic and therapeutic dosages.

The object of this study was to evaluate the effects of a new phosphoorganic systemic parasiticide, administered by different methods at varying dosages, against naturally acquired nematodiasis in beef cattle.

LITERATURE REVIEW

Since the discovery of the systemic insecticidal properties of organic phosphorus compounds by the Germans, investigations have been conducted concerning the effectiveness of such materials as anthelmintics as well as insecticides.

In 1956 Trolene* (Dow ET-57, Dow ET-14, Korlan, Ronnel, O,O-dimethyl, O-2,4,5-trichlorophenyl phosphorothioate) was marketed as a systemically active drug against migrating stages of warbles in cattle. Since the work of Lindquist (1956) many investigators have shown the drug's efficiency against Hypoderma

*Dow Chemical Company, Midland, Michigan.

lineatum (DeVill.) and H. bovis (Linn.). However, as an anthelmintic Trolene does not match its grubicidal qualities. Crenshaw (1956) reported that Trolene at the rates of 125 and 150 mg/kg of body weight would control Haemonchus, Ostertagia and Cooperia in cattle. However, his results were based on treatment of only two yearling calves. Worley (1957) noted that a single dose at the rate of 110 mg/kg of body weight decreased the average EPG (eggs per gram of feces) from 19.4 to 3.2 in wintering Hereford cattle.

Herlich and Johnson (1957) performed critical tests on 8 naturally infected grade Jersey steers treated at the rate of 100 mg/kg. They found that Trolene removed (species followed by per cent) Haemonchus placei 98, Cooperia punctata 59, Ostertagia ostertagi 46, Oesophagostomum radiatum 30, Trichuris spp. 9 and Trichostrongylus axei 0.4. It was not effective against T. colubriformis or Nematodirus helvetianus. Wood (1958) found Trolene ineffective against experimental infections of T. colubriformis in rabbits. Riek (1958) found Trolene to be effective only against H. placei and Cooperia spp. at the rate of 5 g/100 lbs. in calves. He also reported an erratic control of O. radiatum. Riek and Keith (1959) found the same results in similar tests also involving calves. Alicata (1960), using critical tests, administered Ronnel in bolus form (5 g/100 lbs) to 6 calves and found it to have an average efficacy of 79.7 per cent. The calves had been experimentally infected with Cooperia punctata. Drudge et al. (1961) treated beef and dairy cattle with a Ronnel bolus (100 mg/kg) and as a 10 per cent premix at 2 levels (total dose of each, 105 mg/kg) and as a 0.75 per cent spray. Using pre- and post-treatment EPG counts to evaluate the drug's anthelmintic activity, he found that the bolus and premix produced marked EPG reductions while the spray had no effect.

Schad et al. (1958) performed critical tests on range ewes that had been

drenched with Trolene at the rate of 100 mg/kg. They found the drug to be ineffective against Haemonchus, Ostertagia, Trichostrongylus, Nematodirus, Chabertia and the tapeworm Thysanosoma. Similar results were reported by Gordon (1958a) in sheep treated at the rate of 5 g/100 lbs. At twice the dose, he noted that it would remove H. contortus. Based on fecal egg counts, Dorney and Todd (1959) found Trolene, given orally to lambs at rates of 200, 400, and 600 mg/kg, effective against Haemonchus, Ostertagia, Strongyloides, and Nematodirus. Trolene, given in a gelatine capsule at a rate of 100 mg/kg was not effective against an experimental infection of T. axei in 2-month-old lambs (Gibson, 1960).

Levine et al. (1956, 1958a), using horse strongyle larvae, studied the larvicidal activity of several organic phosphorus compounds including Chlorthion, Malathion, Diazinon, DDVP, OS 1808, CoRal, Dipterex and Trolene. Trolene was the only compound inactive at 0.1 Molar (2.5 per cent) concentration. CoRal and Dipterex were larvicidal at 0.0074 M and 0.0001 M concentrations, respectively. However, Kelley and Marsh (1960) found both Trolene and CoRal to be ineffective against migrating larvae of Ascaris suum in baby pigs.

Levine, Kantor and Taylor (1958b) determined the relative toxicities of some of the mentioned compounds in sheep and mice. In mice the LD_{50}'s for DDVP and OS 1808 were fairly low, being 30 and 15 mg/kg, respectively. CoRal at 100-200, Dipterex at 600-800 mg/kg were less toxic, followed by the least toxic of all, Chlorthion at 1000-1200 mg/kg. Two of the 4 nematocides they tested in sheep, OS 1808 and O,O,O-tri-n-propyl phosphorotrithioite, had no effect on the parasites but were toxic to the host. Dipterex given to sheep produced toxic symptoms at a 400 mg/kg dose, however, the animals recovered. Because of the limited amount of DDVP available their results were incomplete

concerning the effective and toxic doses. In limited laboratory tests Riek and Keith (1959) found Chlorthion, Malathion, Diazinon and DDVP ineffective as anthelmintics.

CoRal* (Bayer 21/199, Asuntol, Muscatox, 0,0-diethyl 0-(3-chloro-4 methyl-7-coumarinyl) phosphorothioate), an organic phosphorus insecticide developed by Bayer of Germany and marketed by Chemagro, was approved for use as a grubicide in cattle soon after the appearance of Trolene. As a result of critical tests in cattle and sheep, Herlich and Porter (1958) stated that a CoRal drench at the rate of 25 mg/kg was highly effective against Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Trichuris, Capillaria, and Strongyloides papillosus. At this rate toxic effects were mild and transitory. They also found an erratic control of Nematodirus spp. and Bunostomum phlebotomum. Except for the lack of activity against T. axei and B. phlebotomum, Riek (1958) had similar results in cattle given a dose of 0.25 g/100 lbs. Gordon (1958a) found the drug effective against H. contortus, Trichostrongylus spp. and O. radiatum at the rate of 22 mg/kg but it also resulted in toxicosis and death to a few sheep. Although the drug was effective against parasites in the abomasum and small intestine of sheep, Galvin et al. (1959) lost 13 of 15 animals treated at the rate of 25 mg/kg. In tests with calves he found good control of the intestinal parasites in the same organs with only mild cases of toxicosis. In further tests with lambs and calves, Galvin et al. (1960a) found the drug to be very active against Haemonchus spp. and Cooperia spp. The dosages used were 3-12.5 and 12.5-50 mg/kg in lambs and calves, respectively. Only one lamb showed signs of toxicosis while two calves died at the higher dosages. Dunsmore

*Chemagro Corporation, Kansas City, Missouri.

(1960) injected Bayer 21/199 into the rumen of sheep at 3 g/100 lbs. and found it ineffective against the abomasal parasites. Knight et al. (1960) reported that a 15 mg/kg drench of CoRal was effective against H. contortus, Cooperia spp., T. colubriformis and Nematodirus spp. Eight of 90 sheep involved died of toxicosis. As a 0.25 per cent spray applied to cattle, the drug showed no anthelmintic activity based upon EPG counts (Drudge et al., 1961). Baker et al. (1960), Bailey and Walker (1961) used CoRal at the rate of 20 mg/kg to treat gastrointestinal parasitism in cattle. In both cases the animals which failed to respond to phenothiazine were clinically improved.

Bayer L13/59* (Dipterex, Neguvon, chlorophos, 0,0-dimethyl 2,2,2-trichloro-1-hydroxy ethyl phosphonate), although better known as an insecticide, has been tested as an anthelmintic by several workers. Gordon (1958a) treated sheep with non-toxic doses and found it highly effective against H. contortus. When taken directly into the abomasum it would also remove Trichostrongylus spp. Control of Oesophagostomum radiatum was also observed but it was not dependable. Riek and Keith (1958) found Neguvon at the rate of 2 g/100 lbs. active against H. placei and O. radiatum in cattle. At a higher dose (5 g/100 lbs.) it would also remove Bunostomum phlebotomum, Cooperia spp. and T. axei. They observed that better results were obtained against Cooperia spp. when the drug entered directly into the abomasum. In another experiment Riek (1958) found similar results. Allcata (1960) obtained good control of experimental C. punctata infections in calves by drenching with Bayer L13/59. The drug was safe at the recommended dosages. Dunsmore (1960) also reported that Dipterex was more effective when introduced into the abomasum. Supperer and Pfeiffer (1960)

*Chemagro Corporation, Kansas City, Missouri.

treated calves with Neguvon at the rate of 90 mg/kg and found it highly effective against H. contortus with some activity against Ostertagia spp. In sheep at the rate of 2.5 g/100 lbs. For treatment of acute O. ostertagi infections in young cattle, Banks and Mitton (1960) found that a dose of 69 mg/kg was too low to be effective. However, dosages from 86-140 mg/kg produced excellent results. Watt et al. (1961) used the drug at the rate of 80 mg/kg and found that it reduced egg counts and made the animals more thrifty.

The results of Galvin, Bell and Turk (1959) showed that Bayer L13/59 (5 g/100 lbs.) was effective against abomasal but not intestinal parasites of ruminants. In controlled tests against experimental Trichostrongylus axei infections in 14-month-old sheep, the drug was ineffective as a 100 mg/kg drench (Gibson, 1960).

K'ung et al. (1959) administered Dipterex to sheep and goats by 2 subcutaneous injections. The treatment was effective against Haemonchus, Bunostomum, Nematodirus, Oesophagostomum and Trichuris. However, because of the low worm burdens, the results were suggestive rather than conclusive.

Dimethoate* (Compound CL 12,880, Rogor 40, 0,0-dimethyl S-(N-methylcarbamoylmethylphosphorodithioate) has shown promise as a grubicide, although reports on its possibilities as an anthelmintic are limited.

Drudge et al. (1961) fed dimethoate to cattle as a 10 per cent premix in the grain ration at the rate of 3 mg/kg/day for 5 days. The treatment was discontinued on the fourth day due to toxicosis. There were no significant reductions in the egg counts made at the termination of the test.

Baker et al. (1959) treated Hereford heifers with Dowco 105** as a drench at the rate of 120-130 mg/kg. The results showed that it removed 90-100 per

*American Cyanamid Company, New York, New York.
**Dow Chemical Company, Midland, Michigan.

cent of Trichostrongylus spp., 87 per cent of Cooperia spp., and 66 per cent of Ostertagia spp. Five of the 10 animals used in the test showed toxicosis with one case being severe. Yearling feeder steers with acute parasitic infections which failed to respond to phenothiazine were drenched with a 100 mg/kg dose of Dowco 105 (Baker et al., 1960). The drug proved very effective as judged by the clinical appearance of the animals. Douglas et al. (1959a) tested the anthelmintic activity of the same drug in sheep. The efficacy of the drug against all gastrointestinal parasites in the abomasum and small intestine was 93 per cent at a dosage of 200 mg/kg. When the dosage was reduced to 75 mg/kg the efficacy decreased to 55 per cent. No animals at either dosage showed signs of toxicosis.

Worley (1958) fed yearling feedlot cattle Dowco 109* at rates of 14 mg/kg over 6 and 12 day periods and 15 mg/kg for one day. The drug showed no effect on the intestinal parasites based on egg counts. Dowco 109 differs from Dowco 105 by possessing a methyl rather than an ethyl group attached to the nitrogen atom (Plate 1). Ruelene** is identical to the structure of Dowco 109 except that an oxygen atom replaces a sulfur atom.

Four-tert-butyl-2-chlorophenyl methyl methylphosphoramidate (Ruelene, Dowco 132) has shown promise as an anthelmintic with a broad spectrum of activity and a wide margin of safety.

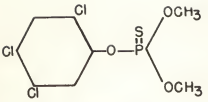
Sewell (1959), Ross and Karr (1959a) compared Ruelene with phenothiazine for worming lambs on pasture. Ruelene at a 150 mg/kg dose reduced EPG counts significantly lower and the lambs gained better than those treated with phenothiazine. The Ruelene treated animals were also more thrifty. In dry

*Dow Chemical Company, Midland, Michigan.
**Dow Chemical Company, Midland, Michigan.

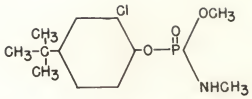
EXPLANATION OF PLATE I.

The structural formulas of some organic phosphorus compounds that have been tested as anthelmintics.

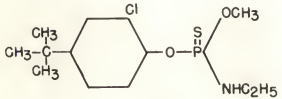
PLATE I



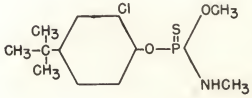
Trolene



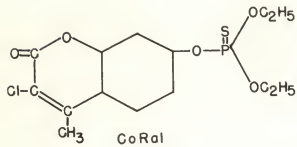
Ruelene



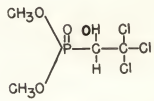
Dowco 105



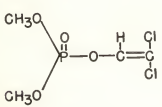
Dowco 109



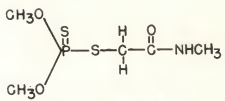
CoRal



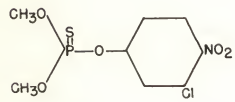
Neguvon



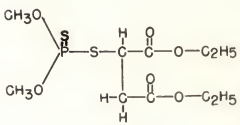
DDVP



Dimethoate



Chlorthion



Malathion

lot experiments Ross and Karr (1959b) compared Ruelene and phenothiazine drenches for worming fattening lambs. The results were similar to the pasture tests as Ruelene produced better gains and lower egg counts. Weight gains of 2-year-old Texas ewes drenched with 2 cc/10 lbs. of either phenothiazine or Ruelene were compared by Ross and Brooks (1960). The Ruelene treated ewes had better gains and showed a thriftier appearance.

In 3-month-old lambs treated with a 200 mg/kg Ruelene drench, Douglas and Baker (1959b) found that Ostertagia spp., Trichostrongylus axei, T. vitrinus and Nematodirus spp. were effectively controlled. The drug was not as active against T. colubriformis. At this rate the drug produced no signs of toxicity. Shaver and Landram (1959) reported that Ruelene as a drench appeared to be effective in sheep, goats, and cattle for the control of Haemonchus, Cooperia, Ostertagia, Trichostrongylus, Oesophagostomum and Strongyloides spp. They suggested dosages of 40, 100 and 125 mg/kg for cattle, sheep, and goats, respectively. At these rates no adverse reactions were observed. Mullison and Shaver (1960) treated cattle with Ruelene at the rate of 40 mg/kg. Five days after treatment the egg counts were reduced by 89 per cent. Administration of the compound as a spray did not affect the EPG's. In sheep treated at the rates of 200, 100 and 50 mg/kg, they found the egg counts reduced by 99, 94 and 93 per cent, respectively.

Kenny (1960) used Ruelene to treat lambs suffering from acute parasitic gastro-enteritis. He drenched the lambs on 2 occasions at the rate of 4 g/100 lbs. The treated animals not only showed much improvement in appearance but the weight gains were almost double that of the controls also. Post-mortem examination of the abomasums of some of the untreated sheep revealed gross numbers of Ostertagia circumcincta and Trichostrongylus axei present in the

ratio of 12 to 1, respectively. Watt et al. (1961) treated bovine ostertagiosis with Ruelene at the rate of 2 g/100 lbs. and found that egg counts were reduced. The animals were also more thrifty. He observed only slight discomfort in four animals due to toxic reactions from the drug.

Galvin, Bell and Turk (1960b) conducted 3 tests involving 30 lambs to determine the anthelmintic and toxic effects of Ruelene. They concluded that Ruelene (100 mg/kg) was not suitable for treatment of mixed infections. They also stated that although higher doses (200 mg/kg) were more active there were signs of toxicosis. Cholinesterase activity was reduced 50 per cent by a 100 mg/kg dose and markedly depressed at higher dosages.

Ioset and Ludwig (1960) reported on preliminary tests using Ruelene as a "pour-on" for intestinal worm control. This method of application showed 89-99 per cent efficiency based upon egg count reduction. Herlich et al. (1961), using the topical application (75 mg/kg), reported erratic control of Cooperia punctata, C. oncophora and Oesophagostomum radiatum, with no activity against Ostertagia ostertagi or Trichostrongylus axei. It was 100 per cent effective against Haemonchus placei. In the same experiment they showed that Ruelene administered orally at dose rates of 40 to 60 mg/kg controlled H. placei, C. punctata, and O. radiatum. At the highest rate it was also active against O. ostertagi, T. axei and T. colubriformis. However, at this rate it was lethal to one of three steers.

Allcata (1960) drenched calves, that had been experimentally infected with C. punctata, with 1.76 g/100 lbs. (39 mg/kg) of Ruelene. The results showed the anthelmintic activity to range from 98.9-100 per cent. No signs of toxicosis were observed in the 100 calves receiving the compound. Drudge et al. (1961) administered Ruelene at the rate of 44 mg/kg as a drench to

cattle in 2 experiments. They found that the treatment reduced egg counts 86 per cent in dairy calves and 97 per cent in Hereford steers.

MATERIALS AND METHODS

Laboratory Diagnostic Techniques

Fecal egg counts were used to estimate the level of intestinal parasite infections in experimental animals. Egg counts were done according to the procedure of the modified Lane (1928) flotation technique of Dewhirst and Hansen (1961). With the use of this technique it is possible to determine the total egg count of each animal which is expressed as eggs per gram of feces (EPG). This method also facilitates the separation of eggs into genera or groups of genera on the basis of morphological characteristics as determined with the aid of a compound microscope. Krug and Mayhew (1949) showed the statistical reliability of the egg classification technique. Hansen and Shivnani (1956) demonstrated the accuracy of the differential egg count method by culturing and identifying infective larvae. Using this method, the eggs were placed in two groups and four genera. One group is the Cooperia, Ostertagia and Trichostrongylus (COT), and the other group, Haemonchus and Oesophagostomum (HO). The eggs of the four genera, Bunostomum, Nematodirus, Trichuris and Strongyloides are quite distinct and easily classified. Strongyloides ova occurred sporadically and are primarily seen in feces of calves so they were not counted.

Although coccidian oocysts were present, no attempt was made to count them or record their presence.

Fecal samples used for making egg counts were obtained directly from the rectum or fresh fecal pads on the ground. The samples obtained from fecal

pads were either dropped by an animal under observation or collected at random from the feedlot. Samples ranging from 5 to 25 grams were collected in small glass jars, numbered and refrigerated at approximately 7°C. until EPG counts could be made. All egg counts were done within two weeks after collection in order to reduce any variation in the count because of storage. Storage results in changes in fecal consistency and morphological characteristics of the eggs (Dewhirst, 1960).

From each sample, 10 grams were weighed into a 300 ml. Erlenmyer flask on a Harvard Trip Balance. Tap water was added to the 300 ml. mark. The entire contents of the flask were poured into the standard 1000 ml. glass container of a Waring Blendor. The samples were homogenized for 30 seconds in the blendor. After homogenizing, approximately 50 ml. were strained into a beaker. As quickly as possible after agitating the beaker, two centrifuge tubes were filled to the 15 ml. mark. This step must be done quickly because the eggs will settle rapidly in water. The tubes were centrifuged for 3 minutes at 1000 r.p.m. in an International Model C50 centrifuge. After allowing the centrifuge to coast to a stop, the supernatant was decanted, leaving the eggs and solid matter packed at the bottom of the tube. The tubes were shaken by hand to break up the packed mass before adding enough aqueous sodium nitrate flotation solution (specific gravity 1.35-1.40) to fill the tubes and form a convex meniscus on top. An 18 mm. square No. 2 cover glass was carefully placed on the top of each tube and the tubes were centrifuged again for 3 minutes at 1000 r.p.m. A Walser Automatic Timer, Model 8066-B, which automatically stopped the centrifuge, was employed to help standardize the technique. After completion of the final centrifugation the two cover slips were carefully removed and placed on a 2 x 3 inch glass slide. The cover slips were

examined, using a standard procedure, under a compound microscope equipped with a mechanical stage. All counts were made using the 10x ocular and 10x objective (low power). The number and type of eggs were recorded on an Adams Laboratory Counter.

For later experiments, the homogenizing and centrifugation times were changed to five seconds and one minute, respectively. The change was innovated because the use of the Waring Blendor for over 10 seconds had been found to reduce EPG counts as much as 50 per cent (Dewhirst, 1960). The centrifugation time was reduced for convenience. In all cases, however, the procedure used was the same for both pre-treatment and post-treatment fecal samples.

Larvae were cultured from three to five grams of feces, spread by means of a tongue depressor, on sterile gauze pads. The pads were placed in eight-ounce wide-mouth bottles and moistened with two ml. of water. Lids were placed on the jars loosely to allow entry of air. After incubation at room temperature (75-80°F.), for 8 to 10 days, each gauze pad was Baermannized, fecal side up, 12 to 18 hours in warm water (85-90°F.). The larvae were collected in shell vials which were corked, numbered and packed in dry ice for shipment to Manhattan, Kansas, from Fort Worth, Texas.

For critical tests, the digestive tract from the abomasum to the anus was obtained from several animals during processing at a packing plant. Each organ (abomasum, small intestine and large intestine) was separated and cut open. The contents of each organ were flushed into a clean 10 gallon garbage can. The gut lining was then scrubbed five or more times by hand. The total washings and organ contents amounted to about five gallons per organ per animal. The contents of each can were allowed to settle 45 to 60 minutes then each can was carefully decanted and refilled with tap water. The

settling, decanting and refilling process was repeated five or six times on the contents of each can. This process disrupts the solid matter and facilitates the concentration of the worms. The final concentration was poured into one-half gallon jars and formalinized. The jars, one for each organ of each animal, were sealed and shipped to Manhattan, Kansas, from the Texas Phenothiazine Company Lab. at Fort Worth, Texas.

The entire contents of each jar were examined under a dissecting scope. A petri dish was marked into sections with a red grease pencil so that each section occupied approximately two-thirds of the low power field. Five to 20 ml. were examined at a time, depending upon the amount of solid matter. The worms were collected with a hooked teasing needle and placed in distilled water for four hours. Then they were placed in 35 per cent alcohol for an additional 4 hours before being transferred to a 70 per cent alcohol-5 per cent glycerol preservative. All worms were identified to genus and to species when possible.

Anthelmintics

In this study, different dosages of Ruelene* (4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate) were administered by various methods to cattle. In only one test, phenothiazine in combination with lead arsenate, was compared with Ruelene.

The Ruelene used for oral treatment (via feed) was in the form of a wettable powder containing 25 per cent active ingredient. The amount of drug needed to meet the proper dosage per animal per day was calculated and weighed out before mixing with the feed ration. In one experiment Ruelene was mixed with the morning ration as a high-level and low-level treatment, 10 and 3 mg/kg

*Dow Chemical Company, Midland, Michigan.

of body weight per day for 5 days, respectively. In a second test, 6.1 pounds of Ruelene formulation M-1839 was mixed with the total 3500 pound ration. The entire 3500 pounds were consumed in 40 hours, which resulted in uptake of the drug at the approximate rate of 25 mg/kg per day or a total dosage of 45 mg/kg per head. About 25 or 1/4 of the animals involved showed excessive salivation 12 to 14 hours after feeding began. One heifer also showed severe respiratory distress. After the second day all animals, including the heifer, had recovered.

The anthelmintic effects of Ruelene as a drench were investigated in tests using dosages of 50 and 75 mg/kg. There were no signs of toxicity in animals treated with the 50 mg/kg dose. However, slight salivation, some stiffness and depression resulted from the 75 mg/kg treatments.

Emulsifiable Ruelene was administered as a "pour-on"* at the rates of 50 and 75 mg/kg. The term "pour-on" describes a new topical application of systemic compounds. This method consists of pouring a measured amount of the drug on a small area of the animal's back.

With the 50 mg/kg "pour-on" treatment no toxic symtoms were exhibited by the animals. However, in one of the tests using the 75 mg/kg "pour-on" about 20 animals showed slight salivation 24 hours after treatment while in other tests the same dose produced no overt symptoms.

Statistical Procedures

Analyses of variance were used to measure the reliability of egg counts performed during the anthelmintic tests. Only for the Crofoot Feedlot experiment was the egg count data transformed according to the formula $\sqrt{x+1}$,

*Dow Chemical Company, Midland, Michigan.

x equals the particular egg count.

A test of repeatability performed on the larval sampling technique revealed that it was reliable.

Chi-square was used to test the validity of differences between EPG and worms post-mortem (WPM) ratios of control and treated animals. Chi-square was also used to measure differences between the number of each species of larvae, before and after treatment, with respect to Ruelene, phenothiazine and the controls.

EXPERIMENTAL RESULTS

Anthelmintic Studies

Cleburne, Texas Feedlot Test (Tables I-II, Plate II, III). Heifer and steer calves of mixed breeds belonging to the Texas Meat and Provision Company, Dallas, Texas, were used in this experiment. The animals, weighing about 300 lbs., were purchased at auctions in central Texas and placed in a feedlot near Cleburne, Texas. Many were treated for shipping fever or pneumonia shortly after arrival. Most of the calves had been in the feedlot from two to four weeks prior to testing.

The cattle were fed from bunkers having a capacity of six tons. The feed was mixed by a local feed company and consisted of ground corn, 29.5; cracked milo, 29.5; cotton seed meal, 13.5; cotton seed bulk, 20.2; ground alfalfa, 5.3; salt and mineral supplement, 2 per cent, and 2 lbs. of Aurofac 10*/ton of mixed feed.

Ruelene was administered topically at 2 rates (50 and 75 mg/kg) and as

*Aureomycin Feed Supplement of American Cyanamid Company. New York, New York.

a 50 mg/kg drench.

Two hundred calves were divided into two pens for the experiment. In the first pen 50 head were tagged in the left ear and treated with Ruelene formulation M-1609 diluted with 2 parts of water. Using a calibrated dipper, about 3.5 oz. of the mixture were applied as a 50 mg/kg pour-on. The remaining 50 head were tagged in the right ear and treated with the same formulation, using about 5 1/3 oz. to provide a dose of 75 mg/kg. In the second pen 50 head were tagged in the left ear and given 3/4 oz. of Ruelene formulation M-1782 using a standard drenching gun. The remaining 50 head were untreated controls. From the treated groups and controls, 12 rectal fecal samples were collected before treatment and again 13 days later from the same animals.

Results of the study based on EPG counts are given in Tables 1-3. Although the treatments reduced the post-treatment egg counts, statistical analyses showed them to be non-significant (Tables 1-2). Grouping the data on the basis of pre-treatment egg counts showed the post-treatment EPG reduction of the treated animals to be highly significant (Table 3).

The animals were slaughtered after they had been fattened. The abomasum (stomach), small and large intestines were recovered from animals 154, 203, 207, 209, 258 (control) and 263 (control). All nematodes present in these organs were collected and identified (Tables 4-9).

The number of worms from the digestive tract of control animals was compared with the number recovered from treated animals that had either similar pre- or post-treatment egg counts (Tables 10 and 11). The worms recovered from treated animals 203 (50 mg/kg drench) were compared with control 263, both of which had similar pre-treatment egg counts (Table 10, Plate II). Control 263 had more than 5 times the number of worms present in 203.

Comparing the organs separately showed that Haemonchus and Cooperia were more susceptible to treatment than other nematodes present. Larval identification performed on a pre-treatment fecal sample of 203 showed that 15 per cent of the eggs present were Haemonchus. Therefore, treatment resulted in 100 per cent control of Haemonchus. Cooperia spp. represented 433 of the 453 worms present in the small intestine of animal 263. The remaining 20 were composed of several genera. Only 35 worms were present in the same organ of animal 203. Twenty-four were Cooperia spp. while the remainder were of several other genera. Thus, the major reduction was in the number of Cooperia present. The worms recovered from treated animals 154 and 207 were compared with control 258 because 154 had a similar post-treatment egg count and 207 had a similar pre-treatment count (Table 11, Plate III). It must be noted that the abomasum from 154 and 258 cannot be compared with each other because of the difference in the nematode populations. However, the nematodes present in the small intestine are similar as to species and number so that a comparison is justified (Tables 4 and 8). Because the egg count of 154 was reduced from 702 to 116, it is reasonable to assume that the number of WPM represents only a portion of the worms present before treatment. The post-treatment count is approximately one-sixth of the pre-treatment egg count. However, this reduction is changed to 1/3 because the egg count of control 258 dropped approximately 50 per cent. Thus, there were 3 times as many worms present before treatment if each egg represented one worm. This is not the case, as the egg-worm ratio of the COT group was 1:13.57 (Dewhirst and Hansen, 1961). Therefore, over 40 times (3x13.57) as many WPM were present in the small intestine prior to treatment. This should be a conservative estimate as this study was conducted on animals in feedlot, whereas, Dewhirst's study (1961) involved animals on pasture.

Table 1. The effect of dermal and oral treatments with Ruelene on egg counts in the feces of Texas cattle.

Treatment	No. Trtd.	No. Smpld.	Average EPG Count & Range 9/13/60	9/26/60	Per Cent Reduction
50 mg/kg pour-on	50	12	216(13-900)	34(4-113)	-84.3
75 mg/kg pour-on	50	12	285(37-709)	75(0-202)	-73.7
50 mg/kg drench	50	12	177(18-1014)	74.5(1-282)	-58.0
Controls	50	12	146(19-440)	117(13-372)	-19.9

Table 2. Analyses of differences between pre-treatment and post-treatment EPG counts, Cleburne feedlot calves.

Source of Variation	d.f.	Ms	F
Treatments	3	56273.16	1.22 ns
Animals:Trts.	41	46293.67	

ns = non-significant

Table 3. Analyses of variance of the grouped EPG data, Cleburne Feedlot Test.

Source of Variation	d.f.	Ms	F
Treatments	3	56273.16	23.52 ***
Animals:Trts:Grps	29	2392.11	

*** $P < .001$

Table 4. Nematodes recovered from animal No. 154.

Organ	Species	Male		Female	Total
Abomasum	Haemonchus spp.	106			106
	Ostertagia♀♀			97	97
	Haemonchus placei			81	81
	Ostertagia ostertagi	62			62
	Cooperia♀♀			28	28
	Haemonchus contortus			17	17
	Trichostrongylus♀♀			13	13
	Cooperia punctata	11			11
	Haemonchus immature		5		5
	Ostertagia lyrata	2			2
	Trichostrongylus axei	2			2
	Subtotals,	183	5	236	424
Small Intestine	Cooperia♀♀			688	688
	Cooperia punctata	438			438
	Cooperia spp.	38			38
	Bunostomum trigonocephalum	3		4	7
	Nematodirus spp.	6		1	7
	Ostertagia ostertagi	1			1
	Trichostrongylus longispicularis	1			1
	Trichostrongylus♀♀			1	1
	Subtotals,	487		694	1181
Large Intestine	Oesophagostomum radiatum	1		4	5
	Trichuris immature		2		2
	Trichuris spp.			1	1
	Subtotals,	1	2	5	8
Totals,		671	7	935	1613

Table 5. Nematodes recovered from animal No. 203.

Organ	Species	Male	Female	Total
Abomasum	Ostertagia ♀♀		40	40
	Ostertagia ostertagi	20		20
	Ostertagia lyrata	1		1
	Bunostomum trigonocephalum	1		1
	Cooperia ♀♀		1	1
	Subtotals,	22	41	63
Small Intestine	Cooperia ♀♀		14	14
	Cooperia punctata	9		9
	Bunostomum trigonocephalum	2	4	6
	Ostertagia ♀♀		2	2
	Strongyloides papillosus		2	2
	Cooperia spp.	1		1
	Nematodirus ♀♀		1	1
	Subtotals,	12	23	35
Large Intestine		0	0	0
	Subtotals,	0	0	0
Totals,		34	64	98

Table 6. Nematodes recovered from animal No. 207.

Organ	Species	Male		Female	Total
Abomasum	Ostertagia♀♀			284	284
	Ostertagia ostertagi	163			163
	Trichostrongylus♀♀			32	32
	Ostertagia lyrata	8			8
	Trichostrongylus axei	7			7
	Ostertagia immature		2		2
	Bunostomum trigonocephalum	2			2
	Cooperia punctata	1			1
	Subtotals,	181	2	316	499
Small intestine	Ostertagia♀♀			20	20
	Ostertagia ostertagi	10			10
	Nematodirus spp.	4		3	7
	Bunostomum trigonocephalum	1		4	5
	Trichostrongylus longispicularis	3			3
	Trichostrongylus♀♀			2	2
	Ostertagia lyrata	2			2
	Cooperia punctata	2			2
	Cooperia♀♀			2	2
	Subtotals,	22		31	53
Large intestine	Unidentified Larvae		5		5
	Oesophagostomum radiatum	1		3	4
	Trichuris spp.			1	1
	Trichuris immature		1		1
	Subtotals,	1	6	4	11
Totals,		204	8	351	563

Table 7. Nematodes recovered from animal No. 209.

Organ	Species	Male	Female	Total
Abomasum	Ostertagia♀♀		257	257
	Ostertagia ostertagi	210		210
	Trichostrongylus ♀♀		161	161
	Trichostrongylus axei	99		99
	Ostertagia lyrata	13		13
	Subtotals,	322	418	740
Small Intestine	Cooperia punctata	5		5
	Cooperia ♀♀		5	5
	Cooperia spp.	2		2
	Ostertagia ostertagi	2		2
	Ostertagia ♀♀		2	2
	Trichostrongylus♀♀		1	1
	Subtotals,	9	8	17
Large Intestine*				
Totals,		331	426	757

*Sample destroyed in shipment.

Table 8. Nematodes recovered from animal No. 258.

Organ	Species	Male		Female	Total
Abomasum	Ostertagia ♂♀			104	104
	Ostertagia ostertagi	84			84
	Trichostrongylus ♂♀			40	40
	Trichostrongylus axei	31			31
	Cooperia ♂♀			22	22
	Cooperia punctata	7			7
	Ostertagia lyrata	3			3
	Haemonchus placei			2	2
	Haemonchus contortus			1	1
	Haemonchus spp.	1			1
	Subtotals,	126		169	295
Small Intestine	Cooperia ♂♀			773	773
	Cooperia punctata	388			388
	Cooperia spp.	114			114
	Bunostomum trigonocephalum	1		1	2
	Nematodirus spp.	1			1
	Ostertagia ♂♀			1	1
	Trichostrongylus longispicularis	1			1
	Trichostrongylus ♂♀			1	1
	Subtotals,	505		776	1281
Large Intestine	Unidentified Larvae		2		2
	Trichuris spp.			1	1
	Subtotals,	0	2	1	3
Totals,		631	2	946	1579

Table 9. Nematodes recovered from animal No. 263.

Organ	Species	Male		Female	Total
Abomasum	Ostertagia ♀♀			18	18
	Trichostrongylus ♀♀			11	11
	Ostertagia ostertagi	9			9
	Haemonchus placei			9	9
	Haemonchus spp.	6			6
	Trichostrongylus axei	5			5
	Haemonchus contortus			4	4
	Cooperia ♀♀			2	2
	Subtotals,	20		44	64
Small Intestine	Cooperia ♀♀			225	225
	Cooperia punctata	176			176
	Cooperia spp.				32
	Trichostrongylus ♀♀			15	15
	Nematodirus spp.	1		1	2
	Ostertagia ♀♀			1	1
	Haemonchus placei			1	1
	Bunostomum trigonocephalum	1			1
	Subtotals,	210		243	453
Large Intestine	Trichuris spp.			3	3
	Trichuris immature		2		2
	Oesophagostomum radiatum	1			1
	Unidentified larva		1		1
	Subtotals,	1	3	3	7
Totals,		231	3	290	524

Table 10. Comparison of the total WPM of a treated and a control animal with similar pre-treatment egg counts.

Animal	Treatment	EPG ***		WPM ***			Total WPM
		Pre	Post	Abo.	Sm.Int.	Lg.Int.	
203	50 mg/kg drench	29	2	63	35	0	98
263	Control	30	82	64	455	7	524

*** P < .001 as determined by Chi-square.

Table 11. Comparison of the total WPM of a control and two treated animals with similar pre- or post-treatment egg counts.

Animal	Treatment	EPG ***		WPM ***			Total WPM
		Pre	Post	Abo.	Sm.Int.	Lg.Int.	
207	50 mg/kg drench	208	21	499	53	11	563
154	75 mg/kg drench	702	116	424	1181	8	1613
258	Control	241	130	295	1281	3	1579

*** P < .001 as determined by Chi-square.

EXPLANATION OF PLATE II.

Comparison of the total worms recovered from each organ of a treated animal and control.

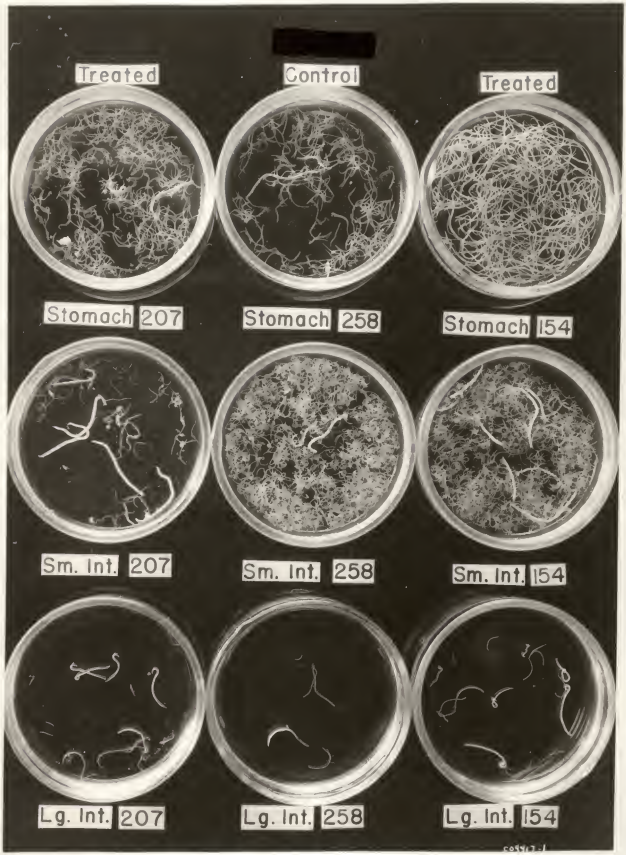
PLATE II


Treated
Control
Stomach 203
Stomach 263
Sm. Int. 203
Sm. Int. 263
Lg. Int. 203
Lg. Int. 263
COTTON-L

EXPLANATION OF PLATE III.

Comparison of the total worms recovered from each organ of a control and two treated animals.

PLATE III


Treated
Control
Treated
Stomach 207
Stomach 258
Stomach 154
Sm. Int. 207
Sm. Int. 258
Sm. Int. 154
Lg. Int. 207
Lg. Int. 258
Lg. Int. 154

Kelly (1955) showed that with an increase in roughage the number of worms per egg increased.

Because of similar pre-treatment egg counts and worm burdens, the number of worms present in the abomasum and small intestine of animals 207 and 258 were compared. Ruelene treatment resulted in 100 per cent control of Haemonchus as larval counts revealed that 8 per cent of the eggs present before treatment were of this genus. Again, as between 154 and 207, there was a great reduction in the number of Cooperia as compared with Ostertagia between animals 207 and 258. The vast majority of worms in the small intestine were Cooperia spp., while in the same organ of the treated animal, 30 of 53 worms were Ostertagia spp. (Tables 6 and 8). This shows that Cooperia spp. are more susceptible to Ruelene than Ostertagia spp.

Crofoot Feedlot Test (Tables 12, 13, Plate IV). Cattle of mixed breed, averaging approximately 800 lbs., from southeastern Oklahoma and northeastern Texas were used in this test. The animals were in the feedlot approximately four weeks before the test began. Some of the animals were treated for shipping fever. The animals were divided and placed in three seperate pens. The animals in 2 of the pens received a high and low dosage of Ruelene at the rates of 10 and 3 mg/kg/head/day for 5 days, respectively. Ruelene, in the form of a wettable powder, was weighed into calculated amounts and mixed with the ration each morning. The animals in the third pen were controls.

The ration was fed from bunkers and consisted of 8 to 9 lbs. grain (50% cracked corn and 50% cracked milo), 1 3/4 lbs. ground alfalfa hay, 8 lbs. sorgo silage and 1 lb. of 41 per cent protein supplement. As feeding progressed the grain was increased to about 22 lbs.

Rectal fecal samples were taken from 20 head that were numbered in each

of the treated lots. Fecal samples from the control animals were taken from 20 fresh pads at random throughout the pen. Samples were taken prior to, 2 days after treatment and twice a month until the animals were slaughtered (Table 12). Fecal samples were taken from the same animals, whenever possible, at each sampling date. Whenever a particular animal could not be sampled, a random sample was taken from the lot so that egg counts could be done on a total of 20 samples from each group. Plate IV shows the curves of the total EPG's at the sampling dates of the treated and control animals.

Analyses of variance performed on the data after transformation showed the low level rate to be just as effective as the high level dosage. Both treatments reduced the EPG counts significantly lower than the controls (Table 13).

Ruelene Versus Phenothiazine-Lead Arsenate Drench Test (Tables 14-17). Ruelene and phenothiazine were administered as drenches to a group of culled calves from the animals used in the Cleburne Feedlot Test. These calves had not been treated. Ruelene formulation M-1782 was given at the rate of 75 mg/kg to 32 head that had been ear-tagged. Two to 3 ounces of a phenothiazine-lead arsenate drench, containing 15 gm phenothiazine and 0.25 gm lead arsenate per ounce, were given to the remaining 36 head. Each group was weighed separately and fecal samples were collected from 10 marked animals in each group. The calves were group-weighed again 16 days later at which time post-treatment fecal samples were collected.

The egg counts of the Ruelene treated calves were reduced 99.2 per cent from the pre-treatment levels while the EPG counts of the phenothiazine treated animals were reduced 61.8 per cent (Table 14). However, the Ruelene treated animals did not gain as well as those receiving the phenothiazine

Table 12. EPG Counts of Crofoot Feedlot Test Cattle.

Treatment	No. Trtd.	No. Smpld.	Average EPG Count & Range 10/20/59	Average EPG Count & Range 12/30/59	Per Cent Reduction
Ruelene 50 mg/kg in feed	201	20	80(3-242)	5(0-21)	-93.6
Ruelene 15 mg/kg in feed	267	20	50(6-407)	6(0-40)	-88.0
Controls	125	20	113(3-512)	50(2-287)*	-55.8

*Control animals were unavailable after December 2, 1959.

Table 13. Analyses of variance in EPG counts of Crofoot Feedlot test.

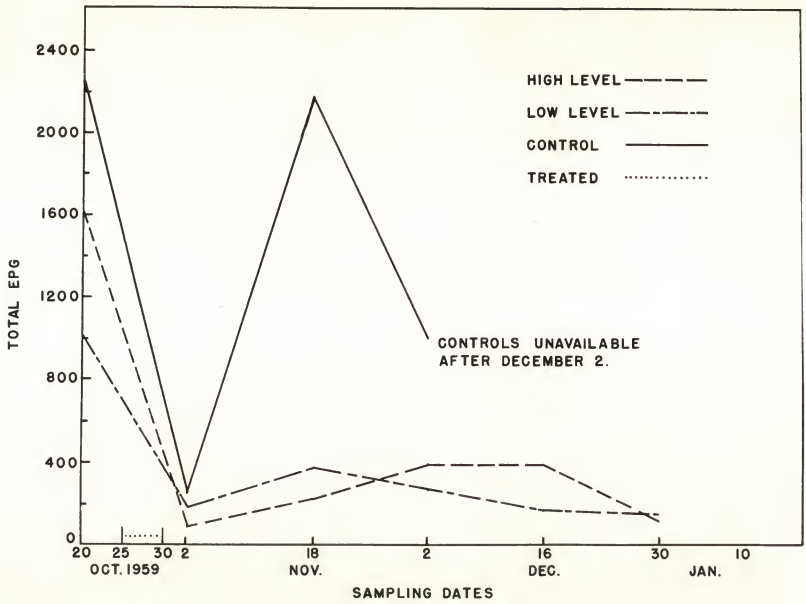
Source of Variation	d.f.	Ms	F
Treatment	2	139.990	10.69 ***
Animals:Trts.	57	18.569	1.42 ***
Periods	3	294.7197	22.50 ***
Periods x Trts.	6	20.8014	1.59 ns
Periods x Animals:Trts.	170	13.0971	- -

*** P < .001

EXPLANATION OF PLATE IV.

Total EPG curves of the animals receiving the high and low level rates of Ruelene and their controls.

PLATE IV


2400
2000
1600
1200
800
400
0
TOTAL EPG
HIGH LEVEL
LOW LEVEL
CONTROL
TREATED
CONTROLS UNAVAILABLE
AFTER DECEMBER 2.
20
25
30
2
18
2
16
30
10
OCT. 1959
NOV.
DEC.
JAN.
SAMPLING DATES

drench which indicates that Ruelene is more adverse in its effects, both against the parasites and the host (Table 15). However, opposite results have been observed in other tests comparing the same drugs (Norris, 1961; personal communication). Because there were no data available on the individual weights of each animal no statistical analyses could be performed.

Analyses of variance of the raw EPG data showed that Ruelene treatment resulted in a higher percentage reduction of egg counts (Table 16). Again, grouping the EPG data into groups (0-299 and 300 up) increased the significance of the differences (Table 17).

Ruelene Oral Versus Dermal Test (Table 18-21). Calves of the same type and quality as those used in the Cleburne Feedlot Test were used to compare feed and pour-on treatments of Ruelene relative to EPG counts.

Eight of 100 head in one pen were tagged in the right ear and fecal samples were taken. The animals in this lot had been taken off feed 12 hours earlier. Ruelene formulation M-1839, using 6.1 lbs., was mixed with the total ration of 3500 lbs. to provide a dosage of 45 mg/kg/head. In another pen of 100 head, 24 were tagged and fecal samples taken. Ruelene formulation M-1609 was administered as a pour-on at the rate of 75 mg/kg to all except 12 of the tagged animals which were controls. Post-treatment fecal samples were again taken from each group 15 days later (Table 18).

Statistical analyses of the EPG data showed that method of administration of Ruelene had no significant effect on the egg count results (Table 19). However, the results approached significance after the EPG data were grouped (Table 20). The difference between the calculated F value of the data and the F value at the .05 level is less in the grouped data than in the non-grouped (Table 21).

Table 14. Experimental design of the Ruelene vs Phenothiazine-Lead Arsenate Test.

Treatment	No. Trtd.	No. Smpld.	Average EPG Count & Range 11/1/60	11/16/60	Per Cent Reduction
Ruelene 75 mg/kg drench	32	9	368(39-851)	3(0-11)	-99.2
Phenothiazine-$Pb_3(AsO_4)_2$ drench	36	10	251(13-842)	86(3-442)	-61.8

Table 15. Ruelene versus Phenothiazine-Lead Arsenate Test.

Treatment	Pre-treatment 11/1/60 Avg. Wt.	No.	Post-treatment 11/16/60 Avg. Wt.	No.	Average Gain (lbs.)
Ruelene	329.37	32	349.31	29	19.94
Phenothiazine*	325.28	36	356.0	10	30.72

*Phenothiazine, 15 gm and Lead Arsenate, 0.25 gm.

Table 16. Analyses of variance in EPG counts of Ruelene and Phenothiazine treatments.

Source of Variation	d.f.	Ms	F
Treatments	1	406725.06	7.79*
Animal:Trts.	14	52241.21	

*P < .05

Table 17. Analyses of variance of EPG data of Ruelene and Phenothiazine treated calves after grouping.

Source of Variation	d.f.	Ms	F
Treatments	1	406725.06	16.34***
Animals:Trts:Grps.	12	24886.56	

*** P < .001

Table 18. Ruelene Oral versus Dermal Test.

Treatment	No. Trtd.	No. Smpld.	Average EPG Count & Range 10/31/60	11/15/60	Per Cent Reduction
Ruelene 45 mg/kg in feed	100	8	126(3-331)	13(0-52)	-89.7
Ruelene 75 mg/kg topical	88	12	206(6-801)	26(1-83)	-88.4
Controls	12	12	353(8-2324)	240(3-1784)	-32.0

Table 19. Statistical analyses of EPG data from the Ruelene Oral versus Dermal Test.

Source of Variation	d.f.	Ms	F
Treatments	2	14350.40	.41 ns
Animals:Trts	27	34737.61	

Table 20. Statistical analyses of the grouped EPG data of the Ruelene Oral versus Dermal Test.

Source of Variation	d.f.	Ms	F
Treatments	2	14350.40	2.88 ns
Animals:Trts:Grps.	19	4979.75	

Table 21. Effect of grouping EPG counts on the F. value.

EPG Data	Calculated F	F at .05	Difference
Non-grouped	.41	3.35	2.94
Grouped	2.88	3.52	.64

Nacogdoches Bull and Heifer Test. Bulls and heifers, weighing an average of 650 and 500 lbs., respectively, were separated into two lots by sex. The animals were numbered and rectal fecal samples were taken before and two weeks after treatment.

Ruelene was administered in varying doses as a pour-on and drench to all animals of both sexes except those designated as controls (Tables 22 and 23).

Although post-treatment EPG counts were reduced substantially, analyses of variance showed that the differences were not significant (Table 24). The data were not grouped because of the small number of animals sampled from each treatment.

Larval Identification

In order to identify the larval stages of cattle nematodes, it is advantageous that they be relaxed (straightened) for measuring. Because it was inconvenient to identify the larvae upon arrival from the Texas Phenothiazine Company Laboratory in Texas, it was necessary to develop a method to simultaneously straighten and preserve them. Hot 70 per cent alcohol or hot formalin proved unsatisfactory because many of the larvae were not straightened. Shivnani and Hansen (1956) applied heat directly to the slide in order to straighten larvae. Because the larvae were to be preserved for later identification, heat was applied by immersing the vials, containing the larvae, into boiling water. Optimum time that the larvae were to be heated was determined by a preliminary experiment using infective larvae of Trichostrongylus axei and T. colubriformis. Twenty seconds proved optimal when using cold fixative (Table 25). Keith (1953) used heat sparingly to avoid shrinkage of the larvae

Table 22. EPG counts of Bull Test.

Treatment	No. Trtd.	No. Smpld.	Average EPG Count & Range 10/29/60	Average EPG Count & Range 11/12/60	Per Cent Change
Ruelene drench	5	4	417(357-486)	93(21-239)	-77.7
Ruelene pour-on	22	12	205(9-1231)	93(9-613)	-54.7
Controls	6	3	352(50-930)	487(42-740)	+27.8

Table 23. EPG counts of Heifer Test.

Treatment	No. Trtd.	No. Smpld.	Average EPG Count & Range 10/29/60	Average EPG Count & Range 11/12/60	Per Cent Change
Ruelene drench	7	6	176(1-430)	20(0-39)	-88.7
Ruelene pour-on	7	3	178(17-365)	66(37-84)	-63.0
Controls	7	6	136(2-589)	141(2-487)	+ 3.6

Table 24. Analyses of variance of the combined EPG data of the Bull and Heifer Test.

Source of Variation	d.f.	Ms	F
Treatments	5	90399.59	2.06 ns
Animals:Trts.	28	43962.87	

Table 25. Relaxing effect of heating time and hot or cold fixative on larvae.

Sample Number	Heating Time	Sample Temperature*		FIXATIVE					
				HOT **			COLD ***		
		Original	Final	No. of Larvae Straight	Total	Per Cent Straight	No. of Larvae Straight	Total	Per Cent Straight
1	5 sec.	29	46	528	618	83.0	-	-	-
2	5 "	28	47	-	-	-	180	545	34.0
3	10 "	28	53	96	114	84.2	-	-	-
4	15 "	28	62	15	15	100.0	-	-	-
5	20 "	27	67	10	10	100.0	-	-	-
6	5 "	29	47	18	42	42.8	-	-	-
7	5 "	28	46	-	-	-	5	75	6.6
8	10 "	28	55	18	18	100.0	-	-	-
9	15 "	28	62	5	5	100.0	-	-	-
10	5 "	31	50	970	1056	91.9	-	-	-
11	10 "	31	56	92	96	96.9	-	-	-
12	15 "	28	61	-	-	-	16	20	80.0
13	20 "	30	69	-	-	-	120	120	100.0
14	30 "	29	74	-	-	-	6	6	100.0
15	40 "	29	80	-	-	-	28	28	100.0

* Centigrade Degrees.
** Same temperature as final of each sample.
*** Room temperature.

within the sheath. Although some shrinkage was noted in the preserved samples, it did not hinder identification. The third stage larvae of Oesophagostomum were the most affected by heat. However, it could be easily identified.

The method of handling the larvae was as follows. After the samples had attained room temperature and the larvae had settled to the bottom of the vial the supernatant was removed with the aid of an eye dropper. Two milliliters of solution, containing the larvae, were left in the bottom of the vial. The supernatant was examined under a dissecting microscope to make sure no larvae had been removed. Fifteen vials were placed in a wire basket constructed to fit inside an 800 ml. beaker. The basket was lowered into the beaker of boiling water for 20 seconds and agitated carefully. After removal from the boiling water, the contents of each vial were doubled by adding cold 20 per cent formalin. This resulted in a 10 per cent formalin solution to preserve the larvae until it was convenient to identify and count them.

The vials containing the preserved larvae were shaken and examined under a dissecting scope to determine the relative concentration of larvae. Larvae were concentrated, when necessary, by decantation of the supernatant. The supernatant was examined in a watch glass under a dissecting scope and any larvae found were returned to the proper vial.

Each vial was agitated by hand for one minute, then three drops of the contents were quickly removed with an eye dropper. The drops were placed in the middle of a 2x3 inch glass slide that had previously been prepared by scratching 2 lines through the center at right angles to each other. A cover glass (18x18 mm. No. 2) was held directly over the center and carefully dropped on the suspension of larvae. Using the vertical line for a reference mark, all larvae up to 25 were counted under the right half of the cover slip.

if 25 were not present under the first half, the larvae on the left side of the reference mark were counted. To avoid counting any larva more than once, each was counted in the field showing the posterior end. All slide samples were carefully examined using the same technique. This procedure was repeated 4 times until 100 larvae were counted from each vial. After every 25 larvae counted, the slide was washed in 70 per cent alcohol and wiped dry.

In some cases the number of larvae present was so small that the sample could not be concentrated enough to provide 25 larvae per 3 drops. When this occurred, the entire sample was examined in a watch glass under a dissecting scope. All larvae were picked up with a small pipette and placed on the slide for identification.

The larvae were identified and counted simultaneously. The number of each species per 25 counted was recorded on an Adams Laboratory Counter. After every 25, the totals were recorded. Identification of the larvae was done according to Hansen and Shivnani (1956) and with the aid of the nomogram by Dewhirst and Hansen (1961). Identification of the larvae served a two-fold purpose in this study, the first being the determination of the relative susceptibility of the various species to Ruelene or phenothiazine treatments, and the second to determine the degree of accuracy of the egg counts.

The larvae of Strongyloides papillosus were easily identified by their long esophagus. However, because the eggs of this species were not counted, neither were the larvae.

The pre-treatment larval samples from Ruelene treated animals were compared with the post-treatment larval counts of the same animals (Table 26). The total larval counts are the sum of five Ruelene treatments from three experiments. The differences between the pre- and post-treatment larval

counts of the Ruelene regimens were highly significant ($P < .001$). Comparison of the larval counts from the control animals of the same experiments revealed no significant changes in the number of each species of larvae (Table 27). Therefore, Ruelene showed activity against certain nematodes present in the test animals.

Ruelene appeared to be most effective against Haemonchus and Cooperia and least active against Trichostrongylus and Ostertagia (Table 28).

Phenothiazine was used in only one experiment. Again, comparisons of the pre- and post-treatment larval counts of the phenothiazine treated animals showed that certain nematodes were more susceptible than others (Table 29). Chi-square showed the differences to be highly significant ($P < .001$), indicating that nematocidal activity of the drug varied among the parasites present. The results of the phenothiazine larval counts are similar to those obtained with Ruelene as the HO group appeared more susceptible, while Ostertagia was the least affected (Table 30).

To determine the accuracy of the differential egg count method employed in this study, the percentages of larvae in the Bunostomum, COT and HO groups were compared with the percentages of eggs for corresponding groups. Only samples that had both EPG and larval counts were included in the percentage calculations. Table 31 compares the larval and EPG counts of cattle used in the Ruelene experiments. The comparisons on the animals used in the phenothiazine experiment were tabulated separately (Table 32). It must be noted that only certain species of larvae can be identified using the method of Dewhirst and Hansen (1961). Nematodirus was not included in the larval counts because the culture period was too short for its development. Therefore, the eggs of Nematodirus were included with those of Trichuris and Capillaria.

Table 26. Comparison of the numbers of each larval species before and after Treatment with Ruelene.

Sample	Species							Total*
	Buno.	C.punct. C.pect.	C.oncho.	Haem.	Oesoph.	Tricho.	Ostert.	
Pre	120	2364	340	892	185	104	579	4584
Post	64	929	43	17	3	250	670	1976

* Chi-square highly significant ($P < .001$).

Table 27. Comparison of the numbers of each larval species in the control groups.

Sample	Species							Total*
	Buno.	C.punct. C.pect.	C.oncho.	Haem.	Oesoph.	Tricho.	Ostert.	
Pre	70	1114	106	336	39	39	282	1986
Post	48	657	58	220	21	30	182	1216

* Chi-square not significant.

Table 28. Susceptibility of some nematodes of cattle to Ruelene treatments determined by larval counts.

Rank	Parasite	Per Cent of Each Species		Net Difference (%)
		Pre-trt.	Post-trt.	
1	Haemonchus	19.4	0.8	-18.6
2	Cooperia onchophora	7.4	2.1	- 5.3
3	C. punctata and C. pectinata	51.5	47.0	- 4.5
4	Oesophagostomum	4.0	0.1	- 3.9
5	Bunostomum	2.6	3.2	+ 0.6
6	Trichostrongylus	2.2	12.6	+10.4
7	Ostertagia	12.9	34.2	+22.3

Table 29. Comparison of pre- and post-treatment larval counts of phenothiazine treated animals.

Sample	Species							
	Buno.	C.punct.: C.pect.	C.oncho.	Haem.	Oesoph.	Tricho.	Ostert.	Total*
Pre	52	417	19	168	8	5	68	737
Post	62	208	0	1	0	21	71	363

* Chi-square highly significant ($P < .001$).

Table 30. Susceptibility of some parasites of cattle to phenothiazine determined by larval counts.

Rank	Parasite	Per Cent of Each Species		Net Difference (%)
		Pre-trt.	Post-trt.	
1	Haemonchus	22.7	0.2	-22.5
2	Cooperia onchophora	2.5	0.0	- 2.5
3	Oesophagostomum	1.0	0.0	- 1.0
4	C. punctata and C. pectinata	56.5	57.3	- 0.8
5	Trichostrongylus	0.6	5.7	+ 5.1
6	Bunostomum	7.0	17.0	+10.0
7	Ostertagia	9.7	19.8	+10.1

The eggs of these genera constituted a group (All Other), which in all cases, was of no consequence in the animals studied.

Error of classification of eggs into COT or HO groups is evidenced by the disagreement between larvae and EPG counts of Groups I, II and IV, given in Table 31. For example, the percentage of larvae and eggs for the COT species, Group I, Table 31, was 72.7 and 91.8, respectively, a difference of 19.1 per cent. The difference between the larval and EPG counts of both the COT and HO species in the Ruelene experiments ranged from 17.3-21.2 per cent, excluding Group III. The other groups could be combined to determine the error because Group III was the only one affected by treatment. The mean difference is 18.75 per cent, which is also the average error. Assuming that the larval percentages were accurate, then 18.75 per cent of the eggs classified as COT should have been classified as HO species. Subtraction of this amount from the COT EPG percentage and addition of the same to the corresponding HO percentage reconciles the differences of Groups I, II and IV of the Ruelene experiments. Using the same calculation procedure, the error for Group III was 0.85 per cent. In the phenothiazine experiment the error was 22.5 and 4.2 per cent before and after treatment, respectively.

DISCUSSION

The evaluation of an anthelmintic is based upon its effect on fecal egg counts supported by critical examination of the intestinal tract. When using egg counts for evaluation of an anthelmintic, technician errors are considered to be consistent throughout the experiment. The activity of a compound is often measured by computing the per cent reduction in the post-treatment egg count. Using the egg reduction criterion only, it is impossible to distin-

Table 31. Comparison of the larval and EPG diagnostic techniques used in Ruelene experiments.

Group	Diagnostic Technique	Species (%)			
		COT	HO	Buno.	All Other
I. (Pre-treatment Test Animals)	Larvae	72.7	24.8	2.5	0.0
	EPG	91.8	7.4	0.6	0.2
II. (Pre-treatment Controls)	Larvae	75.6	21.0	3.4	0.0
	EPG	95.62	3.7	0.6	0.08
III. (Post-treatment Test Animals)	Larvae	96.5	1.3	2.2	0.0
	EPG	97.8	0.9	0.8	0.5
IV. (Post-treatment Controls)	Larvae	74.9	21.0	4.1	0.0
	EPG	96.1	2.5	1.2	0.2

Table 32. Comparison of the larval and EPG diagnostic techniques used in the phenothiazine experiment.

Group	Diagnostic Technique	Species (%)			
		COT	HO	Buno.	All Other
I. (Pre-treatment)	Larvae	69.2	23.8	7.0	0.0
	EPG	91.4	1.0	7.3	0.3
II. (Post-treatment)	Larvae	80.0	0.4	19.6	0.0
	EPG	88.1	0.1	10.3	1.5

guish between actual worm removal and ovulation suppression without the support of critical test data. In the present study the animals were fed from bunkers, thus almost eliminating the chance of reinfection, which justifies the single post-treatment sampling technique. The type of critical testing used in this study did not detect prepatent larvae because they can only be recovered by artificial digestion of organs. Therefore, evaluation of the compounds was based on activity against adult nematodes.

This study revealed that the statistical method employed is important when analyzing EPG data. The analyses of variance of raw data in the study showed that the treatments had no valid effect on the egg counts even though critical tests showed pronounced anthelmintic activity. Values of egg counts had an inherent large variation within test groups which unduly influenced the statistical results between groups. When egg counts were separated into groups of a smaller specific range (0-99; 100-199, etc.), the statistical results were consistent with the results obtained by critical tests (Wood, 1961; personal communication).

Grouping egg counts can be a valuable aid to anthelmintic studies conducted under usual field conditions. In such studies cattle of different breeds, conditions, worm burdens and ages would be encountered. Of course, from the experimental standpoint, it is better to have the treated animals similar to the controls. This would involve setting up the lots of cattle on the basis of pre-treatment egg counts, which is impossible in many cases. Both the treated and control animals should have a similar average and range of egg counts.

The accuracy of the differential egg count method used in this study was determined by comparison of EPG and larval counts. The total counts

were converted to percentages for each species or group because the larval counts were not quantitative as were the egg counts. The error in classifying eggs for the phenothiazine and Ruelene experiments was calculated separately in order to detect any selective action of these drugs against different genera of nematodes. However, the results were the same in both cases. Before treatment there was a considerable error (18.75-22.5%) in both phenothiazine and Ruelene experiments while after treatment the error was markedly (0.85-4.2%) reduced. The increased accuracy in egg classification was related to the elimination of nematodes whose eggs were difficult to classify. This conclusion is supported by two facts. First, results of larval studies showed that the HO group was more susceptible to the anthelmintics used than the COT group. Secondly, the eggs of the HO group are definitely more difficult to classify. Eggs of Haemonchus can easily be mistaken as ova of Ostertagia (Norris, 1960; personal communication).

The results of the egg and larval count comparisons emphasize the fact that larvae are more accurately identified than eggs. However, in most cases the convenience of the less accurate egg count method is preferred to the time consuming larval culturing technique. If the person performing the egg counts is experienced, the error will be at a minimum. The author had performed over 300 egg counts on bovine fecal samples prior to those included in the accuracy determinations. It is difficult to set a definite number of egg counts as the point whereby the technician would be considered experienced. However, in view of the error obtained in this study, 300 should be below the minimum number, if a standard were established.

In order for the novice to gain experience quickly, larval and egg counts should be performed concurrently. Training the technician by this procedure

would increase the accuracy and efficiency of the technique. Establishment of a standard for experienced technicians could then be on the basis of attaining a certain amount of accuracy. Although, in either case, experience is the most vital part in performing an accurate differential fecal egg count.

In all of the conducted tests, Ruelene proved effective against Haemonchus and Cooperia in beef cattle. Comparing the 3 methods of administration, the pour-on technique, with an overall average EPG reduction of 72.4 per cent, was the least effective. Ruelene fed with the ration was the most effective with an average reduction of 90.4 per cent. The average reduction obtained with Ruelene as a drench was 80.9 per cent, although drenching was the least consistent as the reductions varied over a wider range (58.0-99.2 per cent).

A possible explanation for the greater variation obtained when using a drench is the effect produced by the esophageal groove. Several workers have noted a difference in the efficacy of an anthelmintic depending upon whether it was introduced into the rumen or the abomasum. Gordon (1958a), Riek (1958), Riek and Keith (1958) and Dunsmore (1960) all found the anthelmintics they were testing to be more effective when directed into the abomasum. They stimulated closure of the groove by swabbing the pharynx with a 10 per cent $CuSO_4$ solution. According to Dukes (1955) the reflex is evoked immediately after the stimulant comes into contact with the pharyngeal mucous and will last for 15 seconds or longer. Thus, closing the groove and directing the drug into the abomasum results in greater concentration of the compound. If the anthelmintic is swallowed into the rumen, action against the gastrointestinal parasites probably comes through the circulatory system, in the case of systemics, as they feed upon the blood and tissue fluids. Via this route,

concentration of the compound is undoubtedly less than if the drug were introduced directly into the abomasum.

The results of Herlich and Porter (1958) on the efficacy of Bayer 21/199 led them to believe that possibly the anthelmintic action was directly against the worms rather than via the circulatory system. This could possibly be due to a maximum concentration of the drug in the abomasum. Although they did not purposely stimulate the esophageal groove reflex, it may have occurred naturally according to Gordon (1958b). Even though introduction of the nematocide into the abomasum to obtain maximum concentration indicates the possible direct action against the parasites, another alternative should be considered. That is the possibility of the nematodes ingesting greater quantities of the drug from the circulatory system or tissues due to the high concentration of the compound taken directly into the abomasal lining.

Resolvement of the possibilities depends upon the determination of the actual mode of action of anthelmintics of the organic phosphorous type, of which at the present time, much is yet to be learned.

SUMMARY

Studies were conducted on 1,115 beef cattle to determine the efficacy of an organic phosphorous systemic insecticide, Ruelene, against gastrointestinal nematodiasis. The drug was tested at different levels, administered as a drench, pour-on or feed additive. One comparative study included the efficacy of Ruelene versus phenothiazine-lead arsenate.

Ruelene, fed with the ration over a five day period, significantly reduced EPG's by 37.8 and 32.2 per cent more than those of the controls as a high or low level (50 and 15 mg/kg) regimen, respectively.

In one test, Ruelene, as a 45 mg/kg dose fed with the ration, was compared with a pour-on at a 75 mg/kg rate. The EPG reductions were non-significant, although they were 57.7 and 56.4 per cent lower than the controls, respectively.

Bulls and heifers, divided into lots by sex, were used to compare the effects of Ruelene administered as a drench or pour-on. The drench was more effective as it reduced EPG's by 23.0 and 25.7 per cent more than the pour-on in both the bull and heifer tests, respectively.

In direct comparison, Ruelene, given with the feed at the rate of 45 mg/kg, reduced EPG counts by 89.7 per cent while a 75 mg/kg drench reduced egg counts by 88.4 per cent. Controls were down 32.0 per cent.

Administration of a Ruelene drench at the rate of 75 mg/kg reduced egg counts by 99.2 per cent as compared to the 61.8 per cent reduction of a phenothiazine-lead arsenate drench.

In one trial, Ruelene, administered as a 50 mg/kg drench and as a pour-on at 2 rates, 50 and 75 mg/kg, reduced egg counts by 58.0, 84.3 and 73.7 per cent, respectively. The egg counts of the controls were down 19.9 per cent.

Comparison of the critical test data of five animals showed that Ruelene was highly effective against Haemonchus and Cooperia. Little or no effect was evidenced against the other species present.

A method for the mass preservation of straightened infective larvae was devised utilizing boiling water as a killing and straightening agent.

Identification of infective larvae was used to determine the more susceptible species of nematodes to the anthelmintic action of Ruelene or phenothiazine. Haemonchus and Cooperia spp. were the most affected by Ruelene while Trichostrongylus and Ostertagia were the least susceptible. Phenothiazine produced similar results as Haemonchus, C. onchophora and Oesophagostomum

were the most susceptible, while Bunostomum and Ostertagia were the least affected.

Larval identification data were also used to test the accuracy of the differential egg count method. The error in classification of eggs prior to treatment was 18.75 and 22.5 per cent in the Ruelene and phenothiazine tests, respectively. After treatment the error was 0.85 and 4.2 per cent for the same respective drugs. This reduction in error of classification was related to the elimination of nematodes whose eggs were difficult to classify.

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THE EFFICACY OF RUELENE AS AN ANTHELMINTIC IN BEEF CATTLE

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A study utilizing 1,115 beef cattle was conducted to investigate the anthelmintic activity of Ruelene, an organic phosphorus insecticide, and phenothiazine. The experimental design used 193 animals for egg counts and 6 animals for critical tests.

The anthelmintic investigations involved field trials of the drug, varying both the dosage and method of administration. The methods utilized included drenching, pour-on (a new topical application) and feed addition. Ruelene was given at the rates of 50 or 75 mg/kg as a drench or pour-on. When fed as a premix in the ration the rates were 15, 45 and 50 mg/kg in various tests. In most of the tests egg counts were made on fecal samples taken directly from the rectum. In each method of application Ruelene significantly reduced EPG counts. Drenching produced better results than the pour-on, although it was not as consistent. Ruelene produced the highest average egg count reduction when given in the feed. The topical application was the least effective. In a single comparative test, Ruelene as a drench reduced egg counts more than a phenothiazine-lead arsenate drench.

Critical examination of the intestinal tracts of six animals demonstrated the effective anthelmintic activity of Ruelene, particularly against Haemonchus and Cooperia.

Larvae were cultured and identified to determine the nematocidal affect against various species harbored by the cattle. Haemonchus spp. and Cooperia spp. were the most susceptible to treatment while Trichostrongylus spp. and Ostertagia spp. were more resistant.

The larval identification data were compared to the corresponding EPG data to determine the accuracy of the differential egg count technique. Approximately 19 per cent of the ova counted were misclassified.