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Research Article
Levamisole Efficacy on the Helminths of Dog in National Veterinary Research Institute Vom (NVRI) Environment

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Abstract

Background and Objectives: Levamisole is an anthelmintics drug principally use for the treatment and control the range of nematodes in animal and human. It is l-isomer of dl-tetramisole with greater safety margin than the racemic mixture tetramisole. This study was designed to examine the efficacy of levamisole by using modified Mc-master technique. **Materials and Methods:** The experiment was performed on 25 dogs in Vom environment using Modifying Mc-master chamber, polytene bag, concentrated salt solution, microscope, universal bottle and syringe and needle. There were 10 male and 15 female dogs, out of the 25 dogs sampled during the experiment. The organisms identified were Hookworm, Toxocara and Coccidia. The faecal samples were collected directly from both clinical and sub-clinical cases from the rectum labelled and taken to National Veterinary Research institute (NVRI), Vom, Nigeria, Parasitology laboratory for analysis to determine the presence of helminths, eggs and oocyst. The dogs were then treated with levamisole orally and re-sampled after 24 h and analyzed in the NVRI Parasitology laboratory. **Results:** Out of 25 dogs that were sampled 15 are positive while 10 are negative for helminthosis. The results were obtained by comparing the percentage reduction of worm's burden of the first sample collected and the second sample collected after medication. The results were expressed in term of percentage reduction, but from the obtained result showed significant reduction between the first and second samples in hookworm, toxocara ova, but there is no significant reduction in coccidia organism. **Conclusion:** From the study it was reaffirmed that levamisole does not have any effect on the coccidian organism, but it has an anthelmintic property on nematodes such as; hookworms and toxocara organism with some level of resistant in some of the dogs.

Key words: Levamisole, anthelmintics, acetylcholine, toxocara, vermifuge, vermicide, ascaris, nematodes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Levamisole is one of the imidazothiazoles group of drugs as tetramizole, pyrimidine and phenothiazine all of which are cholinomimetic anthelmintics, thus, interferes with the depolarization and contraction of the neuromuscular junctions. Anthelmintic with this mode of action must be very selective in its effect[1]. The bulk of animal ill-health in livestock is brought about by the parasitic activities of helminths, in some cases they cause death which in turn affect human animal protein intake thereby affecting human health[2]. This promote the massive production of synthetic parasiticides and anthelmintics based on the persistent parasitic problem of livestock[3,4]. These drugs are produced in different formulation such as; the injectable, oral and drench preparation[1]. Despite the production of all these drugs and use of same in treatment against helminthiasis in livestock, their administration is very toxic if not administered at the correct recommended dosages[5]. Hence, the need to know the number of worms within the animal gut, the specific diagnosis and finding the characteristic of egg or oocyst in faecal samples[6]. It is often necessary to estimate the output of eggs as a measure of the worm burden[7]. By doing so, the quantity and concentration of eggs can be counted[6].

It is the L-isomer of tetramizole which has the following structure as shown in Fig. 1. Its originally synthesis at Janssen Pharmaceutica resulted in the preparation of a racemic mixture of two enantiomers, whose hydrochloride salt was reported to have a melting point of 264-265°C, the free base of the racemate has a melting point[8] of 87-89°C. The racemic mixture is referred to as "tetramisole"-levamisole refers only to the levorotatory enantiomer of tetramisole. It has a safety factor twice that of tetramizole[1].

It is effective against a wide variety of nematode species for example *Trichostrongylus ostertagia*, *Dictyocaulus*, *Haemonchus*, *Strongylus*, *Strongyloides*, *Rhabditis*, *Ascaris*, *Toxocara* and *Dirofilaria* species[1]. Most of these helminths affect dogs or *Canine* species. Levamisole acts on nicotinic acetylcholine receptor agonist (cholinomimetic) that causes continued stimulation of the parasitic worm muscles and excitatory leading to paralysis at high dosages it also interfere with parasite carbohydrates metabolism by blocking fumarate reductase and succinate oxidase enzymes[1]. By doing so, it inhibit the depolarization and contraction of the muscle[1]. The acetylcholine receptors are located all over the surface of the muscle cells and neuromuscular junctions[9]. Levamisole can be used to treat nematode infestation of both the gastrointestinal and bronchial tract[2]. After being pulled from the market in the US and Canada in 1999 and 2003, respectively, levamisole has been tested in combination with fluorouracil to treat colon cancer. Evidence from clinical trials support its addition to fluorouracil therapy to benefit patients with levamisole efficacy in dogColon cancer[10]. In some of the leukemic cell line studies, both levamisole and tetramisole showed similar effect[11]. Levamisole is a potential candidate for repurposing in medical field, (also termed re-profiling, re-tasking, therapeutic switching or drug repositioning) is the process of developing new indications for existing, failed or abandoned drugs or advanced clinical candidates[10]. In dogs, levamisole hydrochloride (10 mg kg^{-1}) given once daily to a group of dogs produced an adulticidal effect, the filarial mortality was estimated at approximately[1] 60%. The levamisole is used in dogs principally to treat infection with heart worm at doses rate of 10 mg kg^{-1} orally twice daily for 14 days as an adulticide, its absorbed quickly from the gut in dogs but more slowly than suggested by Sandhu and Rampal[1]. Elimination is also rather rapid with mean +l/2 value from 1.3-1.8 h depending on route of administration[1]. It was confirmed that levamisole metabolised by human and canine into aminorex[12]. It excreted rapidly by the canine kidney, over 90% of the dose was recovered in urine in biologically active form which suggested that little metabolic transaction of levamisole occur in dogs[13]. The motivation behind this study to throw a light on the resistance to levamisole by some helminthes of dog and treatment of worms in dogs by using quantitative measure.

MATERIALS AND METHODS

Study area: This investigation was conducted on dogs in NVRI, Vom, Nigeria and its surroundings and it started from April-November, 2019. Vom is located south-east of Jos in Plateau state of Nigeria, with altitude of about 1280 m and annual rainfall of 1,300-1500 mm, extending from April-October and with the months of July and August marking the peak of rainfall. During this period, the temperature ranges from 13.9-31°C. The dry season falls between November and March which is the hottest (31°C). The natural vegetation is mostly grass with short shrubs while there are planted trees around most residential areas[14].

After 8 mg kg^{-1} of levamisole was administered to a dog and faecal samples were collected fresh from the rectum of the animals pretreatment and post-treatment in air-tight bags and taken to the laboratory for analysis[15].

Animal species and breed: The animal species is the canine and breeds vary from the exotic, the local mongrels and their crosses. Twenty four dogs were sampled and their ages ranged from about 3 months to 5 years. There are 10 female and 14 male.

Husbandry system: The management system of most of these dogs is semi-intensive management except for a few under the intensive management.

Experimental design: Faecal samples were collected directly from both clinical and sub-clinical cases from the rectum, without known history of anthelmintic treatment, labeled and taken to the NVRI, Parasitology laboratory analysis to determine the presence of helminthes, eggs, larvae, oocyst and other species of worm's infestation, the dogs were then treated with levamisole and sampled after 24 h.

Materials:

- Muzzle
- Disposable polythene bags
- Concentrated salt solution
- Microscope
- Levamisole tablet and injection
- Universal bottle
- Syringes and needle
- Mc-master chamber or slide

Procedure: The polythene bag was worn on the right hand, the index finger was inserted into the rectum where a reasonable quantity of the fresh faeces was scooped out, faecal samples were collected fresh from the rectum of the animals pretreatment and posttreatment in air-tight bags and taken to the laboratory for analysis. The bag was then turned inside out and tied to prevent contamination. It was labeled then send to the laboratory for analysis, vital information on the individual dogs was collected such as; sex, age and breeds.

Laboratory procedures: The flotation method was used based on the fact that helminths eggs will float in certain solutions in which faecal debris not present, this method was used as qualitative method. An estimated about 3 g of faeces was taken with an application stick and emulsified in the concentrated salt solution. The solution was stirred with applicator stick then sieved into a beaker and poured into a universal bottle. After processing the sample the universal bottle was filled to the brim with the concentrated salt solution. A pasteur pipette was used to suck the surface floated eggs on the surface of the solution and the sucked solution was poured into the counting chamber of Mc-Master slide and both two wells of the slide were filled and left on the bench for g 5 min. Exclude air bubbles from the wells. The slide was mounted on the microscope and examined by using 10 objective lens, count all the eggs within the ruled areas of the two wells. When the sample was analyzed, the animal that tested positive were treated with levamisole at a dose of 8 mg kg^{-1} b.wt. Weight determination was done by using weighting scale and visual examination. The treated animals were re-sampled after 24 h to 3 days to ascertain the level of eggs or oocyst count after medication

Data analysis: The percentage reduction in faecal eggs count was calculated for each treatment dogs using the arithmetic mean of the egg count for each dog after 24 h with the FECR version 4 software. The resistance to an anthelmintic was considered to be present if the reduction (%) in egg count was less than 95% and the lower 95% confidence limit is less than 90.

RESULTS

The structure of levamisole is the l-isomer of dl-tetramisole, it has broad range of anti-nematoda activity in dog (Fig. 1). It acts on autonomic ganglionic stimulant which result in activation of parasympathetic and sympathetic nervous system. It was administered at the dose rate of 8 mg kg^{-1}.

Levamisole efficacy in dog this causes sustained muscular contraction in the susceptible nematodes resulting in muscular paralysis in dog.

The response of worms to levamisole pre and post medication in dogs after 24 h, the post-treatment sampled revealed the percentage reduction and in some cases total disappearance of worms (Fig. 2). Out of 25 dogs sampled 15 are positive to helminthosis viz; 13 dogs are positive to hookworm, 1 positive to toxocara and 1 positive to coccidian organism.

Total number of 25 dogs that were sampled and their feaces examined for gastrol intestinal parasites by using Mc-Master technique, the species of helminthes that are find in sampled dog include: 13 are hookworm, 1 coccidial and 1 toxocara (Table 1).

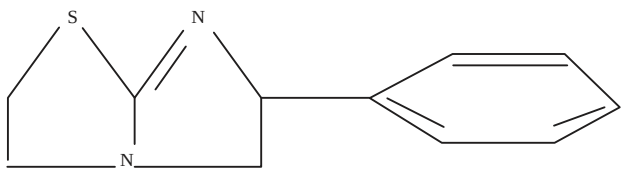

Fig. 1: Structure of levamisole

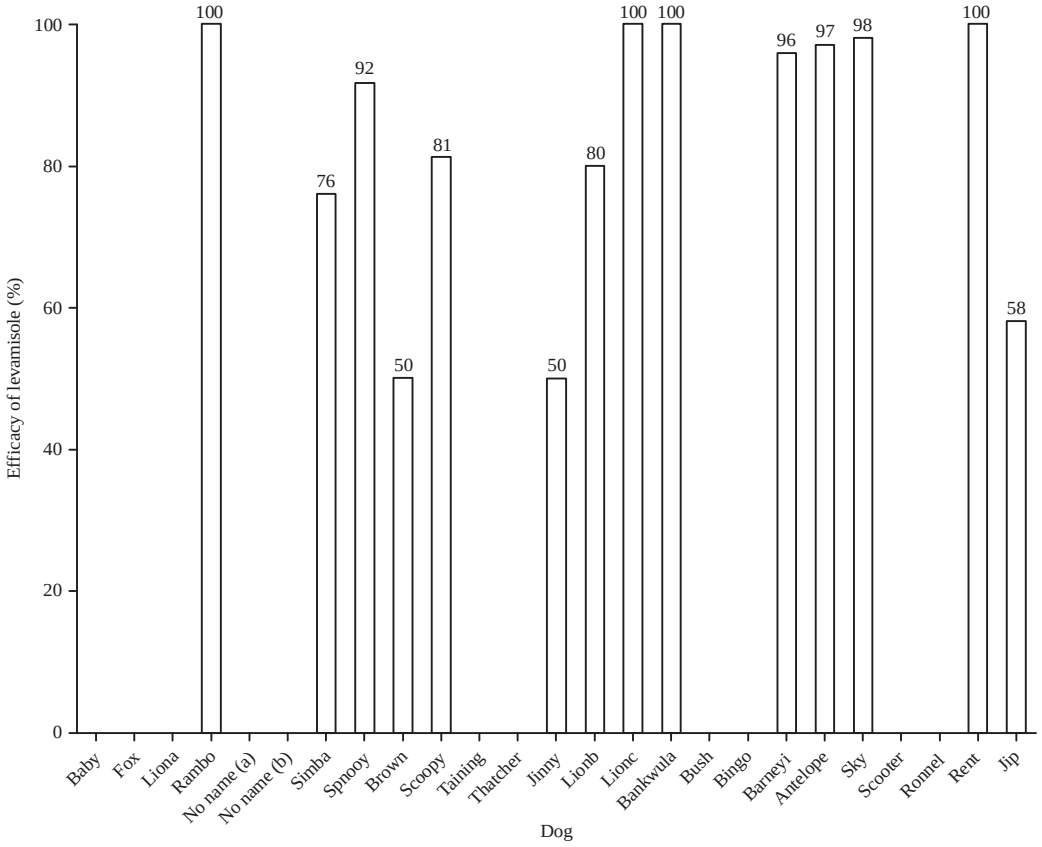

Fig. 2: Efficacy (%) of worms to levamisole at 8 mg kg^{-1} post medication after 24 h in dog

Table 1: Helminthosis (%) in sampling dogs according to species of worms

Species of parasite	Positive number of dogs
Hookworm	13
Toxocara	1
Coccidia	1

DISCUSSION

The study was carried out to evaluate the efficacy of levamisole in helminthes of dog in NVRI, Vom environment. Levamisole (Fig. 1) is the 1-isomer of dl-tetramisole it belongs to the antihelmintic class of medications[16]. The result of 24 fecal sample collected from dog, 15 were positive, out of these positive sample 13 were hookworm, 1 was toxocara species and 1 was coccidia species is agreement with the work of Chidumayo[17]. Figure 2 represent the efficacy of levamisole at the therapeutic dose of 8 mg kg^{-1} has shown maximum response against intestinal parasite such as hookworm and toxocara[1]. It showed a high level of significant reduction rate in most of the worms after 24 h of premedication, is in agreement with previous study by Sandhu and Rampal[1]. While the single dose was used at the present study, the levamisole to have a maximum therapeutic efficacy it's should be administered per os at dose rate of 7.5 mg kg^{-1} daily for 10-30 days[18]. This present study showed that levamisole has no effects on coccidian organisms, is in agreement with previous literature by Borji *et al.*[19] and Holden-Dye and Walker[20]. In this study, indicated that nematode has target sites for levamisole binding such as ion channels and other receptors, which might absence in protozoa which form the bases of non-response by protozoa organism to levamisole anthelmintics[21]. It has been established that some of the dog were frequently dewormed with good anthelmintic routinely in the study area. This was probably due to the proximity and availability of adequate Veterinary Services in the study environment. From the compounds I visited for sample collection, it was noticed that the frequency of deworming was as often as 3 months interval per annum in most cases. With that, one would have expected a very low incidence of gastro-intestinal helminths, but paradoxically the incidence in dogs was still very high. Difference in worms load, management system and breed of dogs used for experiments and level of resistance already developed by helminths are likely reasons for the disparity.

Levamisole is effective against gastro-intestinal nematodes of dogs and is therefore recommended for the treatment of these parasites. It however, has no activity against the coccidia in dogs hence should not be used against such parasites.

CONCLUSION

Levamisole is recommended drug for the control of hookworm and toxocara in dogs which is the most common helminthes problems in Vom and its environs, coccidia organism are not susceptible to levamisole. Dogs get infected as they start growing, the older dogs are more exposed to infection as they roam about but highly resistant than the puppies. Levamisole should be administered by livestock personnel because of toxic effect and to avoid over dose, more so, testing to confirm the efficacy of this drug on the canine species is imperative. The oral route of 8 mg kg^{-1} b.wt., dose for levamisole hydrochloride has proved effective in the treatment of dog's gastrointestinal nematode. This route is, therefore recommended for use in administering levamisole to dogs. Dogs should be dewormed at the beginning, middle and end of the season. To obtain the proper deworming using levamisole, the treatment should be continued for not less than 10 days with that recommended dose.

SIGNIFICANCE STATEMENT

This study reaffirmed the efficacy of levamisole and its resistance against hookworm and toxocara using quantitative measure and it's also points out how ineffective of levamisole against coccidian organism. The quantitative assessment of the sample using the McMaster method is an extra benefit for a clinician to evaluate the efficacy and resistance of a specific anti-parasitic therapy if require. This study will also help the researchers, livestock farmers, dog breeder to uncover the critical areas of helminthosis and to explore the management programs for dog critical areas of deworming schedule.

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