Anthelmintic efficacy of Fenbendazole and Levamisole in native fowl in northern Iran

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Research

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Abstract

Background: With the increasing number of free-range domestic chickens, helminth parasites have potentially become more of a threat in commercial flocks in recent years, and routine poultry deworming is needed to improve efficiency of chicken products. The present study deals with a field trial to study the efficiency of two generally used anthelmintics, Fenbendazole and Levamisole, against gastrointestinal nematodes of domestic chickens in north of Iran.

Methods: Out of 45 domestic chicken flocks involved in the study, 20 flocks were selected to participate in fecal egg count reduction testing based on flock size from April 2017 to September 2018. The infected chickens were randomly divided into three equal groups of 30 each. Ninety chickens in the infected groups received one of the following treatments (d 0 of treatment): Group 1: 5 mg kg−1 body weight (BW) Fenbendazole for three consecutive days, Group 2: 16 mg kg−1 BW Levamisole, and Group 3 control: placebo; water + DMSO [1]. The efficiency of the treatments were evaluated by comparing fecal egg count in the treated and control groups.

Results: Examination of 3 herds of chicken from the control group showed that 95.0% of the animals were infected with gastrointestinal nematodes with an average geometric value of 361 eggs per gram of feces. Fenbendazole at a dose of 5 mg kg−1 BW for three days showed an efficiency of 83.7% ($P \geq 0.05$), and Levamisole at a dose of 16 mg kg−1 BW showed 71.8% ($P \geq 0.05$) with geometric mean eggs in a gram of feces 100 and 199.6, respectively. In general, Fenbendazole and Levamisole showed significantly lower activity. The result of this study revealed that Fenbendazole was a better and effective dewormer than Levamisole on the three Iranian domestic chicken flocks, but it is not significant. *Capillaria* spp., were the most generally resistant nematodes followed by *Trichostrongylus* spp., and *Amidostomum anseris*.

Conclusion: Our results indicated that Fenbendazole and Levamisole reduced number of nemathodes effectively in three Iranian domestic chicken flocks. Given the results of our study, it is possible that resistance may be expected in the parasitic helminths of poultry. Additional studies with a larger scale are required to determine the prevalence of anthelmintic resistance in the poultry industry.

Introduction

Domestic chickens play important role in the economics of rural regions of small societies all over the world. Traditional free- range chickens provide a critical source of food and income for the people living there. However, the growing request on chicken products from free- range production systems is hampered by the heavy burden of diverse poultry helminths reemerging [1]. Helminth parasitism remains one of the most significant widespread infestations in rural chicken worldwide due to scavenging habits in free- range raising chicken, persuading clinical and subclinical disease, which impends food production and chicken health. Consequently, practical parasite control approaches are required to withstand poultry health and production, and hence, to confirm the sustainability of poultry products. Not surprisingly, the

control of gastrointestinal parasites in poultry is restricted by the high cost of anthelmintics, indefinite availability, and the growing occurrence of drug resistance.

In Iran, three broad-spectrum anthelmintic classes with different modes of action are listed for practice in livestock; the imidazothiazoles (e.g., Levamisole), the macrocyclic lactones (e.g., ivermectin), and the benzimidazoles (e.g., Fenbendazole). From the time when the announcement of the benzimidazole drugs in the 1960's, it has been used broadly in a wide range of species for the control of parasitic helminths. These anthelmintic compounds represent inarguably the most extensive therapeutic effect against diverse parasites species, excellent anti-parasitic efficiency, and very little toxicity in treated animals. Fenbendazole is one of the safest drugs used in food animals in terms of food residues[2]. This is principally essential in the poultry industry where benzimidazoles are veterinary drugs extensively used for treatment and prevention of parasitic infestations. Levamisole is one of the imidazothiazole derivatives that is a potent and efficient anthelmintic for gastrointestinal nematode infestations in poultry, companion animals, and swine, and also against lungworms[2]. Following the encouraging findings in the poultry industry, Levamisole is generally used for the prevention and treatment of ascarid infections [3].

In recent years, anthelmintic resistance is a prevalent problem for the control of gastrointestinal nematodes of animals in virtually every region of the world [4] [5, 6]. In Iran, studies assessing the field efficiency of anthelmintics and the detection of anthelmintic resistance have been performed since 2007[7]. Anthelmintic resistance (AR) in Iran has been described in gastrointestinal nematodes of sheep (benzimidazole and Levamisole resistance)[7-12] and horse (benzimidazole)[13]. It should be noted that some evidence on AR was not accessible due to inevitable conditions (e.g., information not released for consideration by scientists, etc.). Moreover, these reports indicated a partial amount of selected farms, and no additional supposition could be concluded for parasitic drug resistance at a local or national level. On the other hand, until now, no former effort has been tried to perform the field efficacy of anthelmintics in poultry. Consequently, the present study was conducted to assess the *in vivo* anthelmintic efficacy of Fenbendazole and Levamisole against the gastrointestinal helminths of chickens in the north of Iran.

Material And Methods

Study area

This study was conducted in naturally infected flocks of domestic chickens in Sari city in northern Iran (longitude 51°26' E, latitude 35°41' N) from April 2017 to September 2018. Sari is the provincial capital of Mazandaran province, located in the north of Iran, between the northern slopes of the Alborz Mountains and the southern coast of the Caspian Sea (Fig.1). Sari has a humid subtropical climate, with a Mediterranean climate influence. It lies approximately 32m above sea level and has a mild climate characterized by rainfall of 789.2 mm per annum. The mean annual temperatures are 15°C.

Selection of flocks

Out of 45 domestic chicken flocks allocated into the study, 3 flocks were chosen to participate in fecal egg count reduction testing (FECRT) based on flock size. The following criteria were used: (i) the flock had 40 - 60 domestic chickens of different ages and sexes; (ii) anthelmintic drugs had not been used within past eight weeks; (iii) average fecal egg counts (FEC) were higher than or equivalent to 100 eggs per gram (EPG) of feces; and (iv) there was a history of anthelmintic application on the flock in the last three years. When a farmer approved to take part and met the first two principles, fecal samples were collected from twenty randomly selected domestic chickens and tested for FEC. Over 45 flocks were tested to obtain 3 suitable flocks for the FECRT trial.

During the experiment, chickens were kept indoors and fed according to the norms of the field and water was ad lib. Three to four daysbefore the experiment was conducted, feces were examined using the McMaster method, then domestic chickens were randomly assigned to experimental groups with a similar degree of infection to further determine the geometric mean number of eggs per gram of feces.

Fecal egg count reduction test (FECRT)

The FECRT was done for each flock, according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for the assessment of anthelmintic efficacy in poultry (14). The ninety infected chickens were randomly distributed into three equal groups of 30 each. Each group was kept in a separate wire-floored cage (550 cm2/chicken).The chickens, naturally infected with helminths, were randomly divided into 3 experimental groups as follows with 10 chickens in each and one group was considered as the control.

Group 1: 5 mg kg−1 body weight (BW) of Fenbendazole for 3 consecutive days

Group 2: 16 mg kg−1 BW of Levamisole

Group 3: placebo; water + DMSO (the control).

Drugs doses were calculated based on a recent study on the efficacy of the Fenbendazole and Levamisole, against internal parasites in chickens [15, 16]. To standardize the doses of Fenbendazole and Levamisole all chickens were treated individually as the doses were given orally via syringe with a blunt needle.

2.3.4. Fecal egg counts (FEC)

Fresh fecal samples were collected from a random sample of chickens (30% of each infected group). Selected chickens were marked, and only on the day of sampling each chicken was placed into an individual cage for defecation. Three grams of fecal samples were collected from the initially selected chickens d 0 (for pre-treatment FEC) and d 14 post-treatment into zip-lock plastic bags, and were kept at 4 °C until processed for FEC using a modified McMaster technique [17]. A decrease in % of FEC describes the percent of reduced FEC between negative control and treated groups [17]. It was calculated on d 14 post-treatment according to the following formula:

% FEC reduction test (FECRT) =100× (FEC control−FEC treated) FEC control

Necropsy

Lastly, for postmortem examination, chickens were euthanized and dissected on day 14 after dosing to obtain worms [18]. Immediately, the gastrointestinal tract of each chicken was removed, and the small intestine was separated. The contents of the intestine was washed into a sieve of 100 μm pore size and inspected for the presence of adult helminths. Subsequently, all visible worms were collected. Evaluation of anthelmintic efficacy was calculated consistent with the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) for assessing the efficiency of anthelmintics in turkeys and chickens [14]. The efficiency of the treatments was evaluated by comparing FEC in the treated groups and control group.

Statistical analysis

Statistical analysis was performed with SPSS 19.0 software (SPSS Inc, Chicago, IL, USA) with a P value of <0.05 as statistically significant. Repeated measures ANOVA was used to compare the differences between the experimental and control groups of animals.

The effectiveness of anthelmintic drugs was determined by the “control test” method according to the results of coproscopic examinations of animals with the calculation of the geometric mean value of the number of helminth eggs in samples before and 15 days after deworming of animals from the experimental and control groups. The independent samples T-test was uses to compare the efficacy of two anthelmintic drugs.

Interpretation of the FECRT results

Anthelmintic resistance status was understood as suggested by the WAAVP guidelines on AR based on the percentage of fecal egg count reduction (%FECR) and the upper (UCL) and lower (LCL) 95% confidence limits [18]. Therefore, each anthelmintic was confirmed as (i) effective when the %FECR and UCL were both ≥ 95% and the LCL was ≥ 90%, (ii) suspected resistant when %FECR was < 95% or LCL was < 90%, and (iii) ineffective/resistant when both %FECR was < 95% and LCL was < 90%.

Ethical statement

The blinded, randomized, and placebo-controlled study was performed according to the guidance for the experimental study of pharmacological substances. The use of domestic chickens in this study was approved by the Animal Ethics Committee (AEC no. 45403.3.1) of the Ferdowsi University of Mashhad.

Results

Examination of 3 herds of chickens from the control group showed that the animals were infected with gastrointestinal nematodes (GINs) by 95.0% with an average geometric value of 361 eggs per gram of

feces. Fenbendazole at a dose of 5 mg-kg BW for three days against GINs showed an efficiency of 83.7% (P ≥0.05), and Levamisole at a dose of 16 mg-kg BW showed 71.8% (P ≥0.05) with geometric mean eggs in a gram of feces 100 and 199.6 for Fenbendazole and Levamisole, respectively . In general, both Fenbendazole and Levamisole showed low activity against GINs

The FECRT results of this study showed that there was no significant difference between the groups treated with Fenbendazole and Levamisole with the control group. Also, there was no significant difference between the EPG of the control group and the treatment groups at the beginning of treatment.

The FECRT results revealed that Fenbendazole was more effective (83.7%) than Levamisole (71.8%), but the difference was not significant (Fig.2). The pre-treatment egg identification of GINs from all flocks of domestic chickens showed mixed GIN infections containing *Capillaria* spp., *Ascaridia galli/ Heterakis* spp., *Syngamus trachea*, *Trichostrongylus spp.*and *Amidostomom anseris*.

Capillaria spp. were the most commonly resistant nematodes, followed by *Trichostrongylus* spp. and *A.anseris* (13%) whereas *A. galli/Heterakis spp.* and *S. trachea* were susceptible to anthelmintics examined in this study.

Necropsy and worm burden of GINs in pre- and post-treatment groups of domestic chickens showed that Fenbendazole was more effective to remove all the GINs (i.e., *A. galli, Heterakis spp.* and *S. trachea*.) prevalent on 20 domestic chicken flocks. Fenbendazole did not affect *capillaria* spp. and *Trichostrongylus* spp., with minimal effect on *A.anseris*. Likewise, Levamisole seemed to have the highest efficacy against *S. trachea* while it was less effective against other GINs.

Discussion

Despite the constant development in housing and management measures in poultry breeding, chickens kept on deep litter often must be treated for parasitic infections. The present study deals with a field trial to investigate the efficacy of two commonly used anthelmintics as Fenbendazole and Levamisole against GINs of domestic chickens. Our results indicated that there is a reduced efficacy in Fenbendazole and Levamisole in three Iranian domestic chicken flocks. However, the results of this study revealed that Fenbendazole was more effective anthelmintic than Levamisole in the three Iranian domestic chicken flocks but the difference was not significant. *Capillaria* spp. were the most generally resistant nematodes followed by *Trichostrongylus* spp., and *A.anseris*.

Previously, AR in GINs of sheep[7] and horses[13] had been reported from Iran against benzimidazole compounds, but no previous data has been published on the field efficacy of benzimidazole compounds in poultry.

Various information is available on the therapeutic doses of Fenbendazole and Levamosole in chickens. However, the FECRT results of this study should be interpreted carefully, as we tested this two anthelmintics in domestic chickens based on the dose rates recommended in previous studies [15, 16].

Fenbendazole and Levamisole are widely used broad-spectrum anthelmintic drugs in sheep, horse and cattle in Iran. Anthelmintic resistance to BZs and Levamisole has been reported for GINs of sheep from Iran [11]. Recently, Mohseni et al. [12] conducted a regional survey to evaluate the prevalence of AR in GINs of sheep in northeast of Iran and they found an average efficacy of 46% and 44% for albendazole, and Levamisole, respectively. Similarly, we found that Fenbendazole was more effective (83.7%) than Levamisole (71.8%). The reason for the reduction in sensitivity to Levamisole in poultry flocks may be due to the frequent routine treatments which impose strong selection pressure on worm populations and encourage the development of resistant strains. Due to the fact that only three major chemical groups are currently available for the treatment of gastrointestinal and pulmonary nematodes, it is imperative that their usefulness is conserved for as long as possible.

There are three main helminths that usually affect chickens - these are gapeworms, roundworms, and tapeworms. Fenbendazole and Levamisole are generally used for the control of three species of helminth parasites of chickens including *A. galli*, *Heterakis gallinarum,* and *Capillaria* spp. which has no adverse effect on the poultry eggs-laying or hatching[2]. Of the species found in domestic chickens, *A. galli,* heavily parasitizes the free-ranging poultries [19, 20]; hence, control measures such as preventing infections or chemotherapy may improve weight gain and egg production. The results of our study revealed that Fenbendazole and Levamisole were more effective in eliminating *A. galli*. It should be noted that evidence of Fenbendazole resistance in *A. dissimilis*, a closely related ascarid of chickens, has been recently reported in turkey with a history of heavy administration of frequent intervals of Fenbendazole [21].

Moreover, *H. gallinarum* has been reported as another most frequent species of helminths in domestic chicken in Iran[19], which Fenbendazole and Levamisole are highly effective against it. Additionally, the *Syngamus trachea*, which is a parasitic nematode that infects the tracheas of individual birds, is also common in young and domesticated chickens[22].

On the other hand, among other nematodes, Fenbendazole and Levamisole do not affect *Capillaria* spp., which affects many species of wild birds and domesticated poultry, turkeys and ducks[22]. The signs of infection with *Capillaria* spp. can be challenging to identify due to the chronic nature of infection, and some non-specific signs include intermittent diarrhea, reduced appetite, ill thrift, and weight loss[18]. When *Capillaria* spp. are present in high numbers, it can be fatal to the chicken[22]. Given the ubiquity of *Capillaria* spp. and its possible economic impact, parasite control is an essential issue for bird health and productivity. Furthermore, *Trichostrongylus tenuis,* which is a gut nematode found in Iranian domestic chicken has not sensitivity to Fenbendazole and Levamisole. This endoparasite can cause poor condition and reproduction, leading to a condition often called strongylosis or grouse disease[22].

The finding of resistance to Fenbendazole and Levamisole in *Capillaria* spp. and *Trichostrongylus* spp. may have far-reaching consequences for the poultry industry, both in terms of animal welfare and economic impact. Given the results of our study along with the former finding of the suboptimal efficacy of Fenbendazole in *A. dissimilis* [21], it seems likely that resistance may be expected in the helminth

parasite of poultry. Subsequently, more extensive scale surveys for resistance are needed to determine the prevalence of anthelmintic resistance in the poultry industry. Furthermore, studies addressing the production costs of drug-resistant *Capillaria* spp. are required to determine the financial impression on the industry.

Declarations

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Availability of data and materials:

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Authors' contributions

Borji H. conceived the idea and provided feedback on the manuscript. Borji and Kalidari designed the study. Saemi conducted the field work. Saemi conducted laboratory work, and Borji analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

Notes

Ethics approval and consent to participate

The use of domestic chickens in this study was approved by the Animal Ethics Committee (AEC no. 45403.3.1) of the Ferdowsi University of Mashhad.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table1

Data on farms included in the fecal egg count reduction test in the domestic chickens in the north of Iran from April 2017 to September 2018

farm	Group	Group size	Group mean EPG pre	Group mean EPG post	Max individual EPG prec	Max individual EPG postc	Efficacy mean of drug
1	1(control)	5	215.8	212.6	625	606	-
	2(fbz)	5	251.8	59.2	583	134	79.38%
	3(lev)	5	398.8	213.8	602	525	43.81%
2	1(control)	7	141.7	139	337	332	-
	2(fbz)	7	137.6	22.9	263	85	85.18%
	3(lev)	7	326.7	137	628	38	89.81%
3	1(control)	5	84.4	89.4	149	147	-
	2(fbz)	5	178.8	29	294	81	86.68%
	3(lev)	5	120.4	15.2	218	36	81.82%

Table2

The helminth egg species observed in the domestic chickens in the north of Iran from April 2017 to September 2018

farm	1			2			3		
	cont	fbz	lev	cont	fbz	lev	cont	fbz	le
The species of helminth egg observed pre treatment	*Cap* *Asc* *Trich* *Amid*	*Cap* *Asc* *Trich* *Amid* *Syn*	*Cap* *Asc* *Trich* *Amid* *Syn*	*Cap* *Asc* *Trich* *Syn*	*Cap* *Asc* *Trich* *Amid* *Syn*	*Cap* *Asc* *Trich* *Syng*	*Cap* *Asc* *Trich* *Amid*	*Cap* *Asc* *Trich* *Amid*	*Cap* *Asc* *Tric* *Ami*
The species of helminth egg observed post treatment	*Cap* *Asc* *Trich* *Amid*	*Cap*	*Cap* *Trich*	*Cap* *Asc* *Trich* *Amid*	*Cap*	*Cap*	*Cap* *Asc* *Trich* *Amid*	*Cap* *Trich* *Amid*	*Cap* *Tric* *Ami*

cont: control

fbz: fenbendazole

lev: levamisole

Table3

Necropsy results in farm 2 after treatment in the domestic chickens in the north of Iran from April 2017 to September 2018

group	Mean efficacy of drug	The helminth species observed post treatment in farm2
control	-	*Ascaridia galli* *Symgamus trachea* *Capillaria obsignata*
fenbandazole	83.7%	*Capillaria annulata* *Capillaria obsignata*
levamisole	71.8%	*Postharmostomum gallinarum* *Raillietina cestode*

Figures

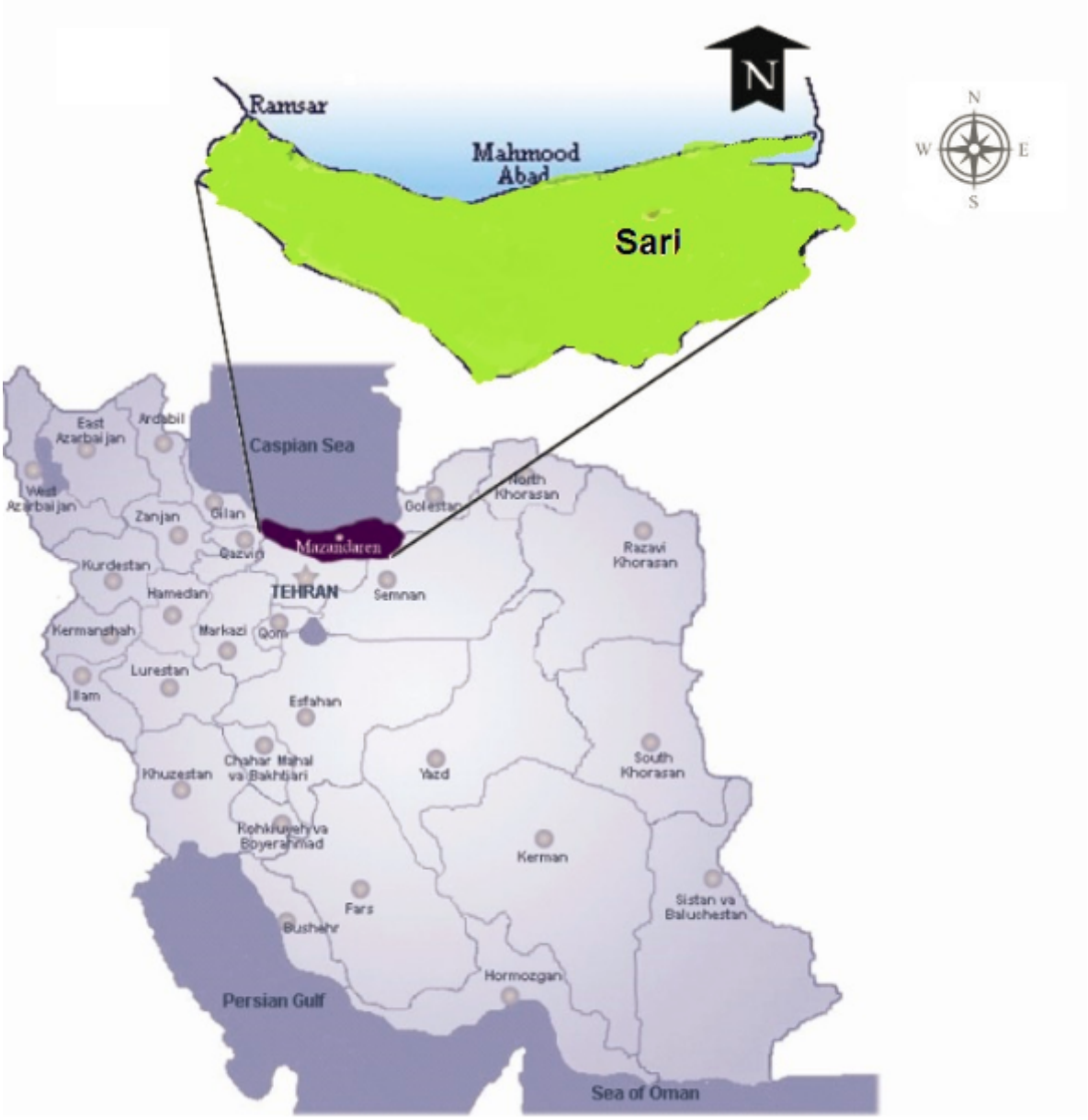

Figure 1

Map of Iran, the highlighting position of Sari City in Mazandaran province Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Image not available with this version

Figure 2

The proportion of farms with resistance, suspected resistance and susceptibility of gastrointestinal nematodes of domestic chickens to two anthelmintics on 20 farms in Iran.