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## Comparative efficacy of seven brands of albendazole against naturally acquired gastrointestinal nematodes in sheep in Hawassa, southern Ethiopia

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**Abstract:** This study aimed to evaluate the efficacy of 7 brands of albendazole against gastrointestinal nematodes in naturally infected sheep in southern Ethiopia. The study included 120 local breed male sheep purchased in Hawassa. The sheep were divided into 8 groups of 15 animals each. Group 1 served as the untreated control, whereas groups 2-8 were treated with ABZ<sub>1</sub>, ABZ<sub>2</sub>, ABZ<sub>3</sub>, ABZ<sub>4</sub>, ABZ<sub>5</sub>, ABZ<sub>6</sub>, and ABZ<sub>7</sub>, respectively.

Fecal samples were collected before treatment on day 0, and again on post-treatment day 12. The efficacy of all 7 brands of albendazole was determined on post-treatment day 12 based on the fecal egg count reduction test (FECRT). The results of the study show that the efficacy of 5 brands was good, whereas that of the other 2 brands was low. The observed differences in efficacy between the brands of albendazole were most likely due to variations in quality rather than the administered doses. Coprocultures from all pre- and post-treatment samples showed a predominance of *Haemonchus* spp. Results of a questionnaire survey indicated that the benzimidazoles are the most widely used anthelmintic family, followed by the imidazothiazoles and macrocyclic lactones. In addition, it showed that farmers in the study area were engaged in several practices that may be responsible for lowering the efficacy of anthelmintics. Additional detailed studies are required to clarify the current status of the efficacy of the anthelmintics widely used in different agroecologies, animal species, and livestock management systems in Ethiopia.

**Key words:** Albendazole brands, efficacy, sheep, helminths, Hawassa

### Introduction

Ethiopia is home to 23.6 million sheep and 23.3 million goats (1); however, the immense potential these numbers represent has yet to be realized due to a multitude of factors. Among the major hindrances to the productivity of small ruminants, helminthosis results in enormous economic losses, especially in

areas where ruminants are kept on pasture throughout the entire year, such as Ethiopia (2).

Parasitic helminths are a primary factor in the reduced productivity of small ruminants in many parts of the world, particularly in developing countries where nutrition and sanitation are generally poor. Infection by helminths negatively affects sheep

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productivity and leads to stunted growth, reduced weight gain, poor feed utilization and conversion, low fertility, condemnation of affected organs, high treatment costs, and mortality. The chronic insidious form of helminthosis is much more common, and is associated with greater economic losses than acute diseases and many other lethal infectious diseases (2,3).

Studies show that up to 95% of sheep and goats in the tropics are infected with helminths. In tropical countries gastrointestinal nematodes are responsible for a 23%-63% reduction in growth (3), up to 25% of pre-weaning mortality (4), and 24%-47% of reductions in annual offtake from small ruminants.

Several abattoir and coprological studies indicate that infection by nematodes in small ruminants is highly prevalent and widespread in all agroecologies and livestock production systems in Ethiopia (7-9). Approximately 80% of the Ethiopian sheep population is reported to harbor varying degrees of infection with different parasitic nematode species. Due to the lack of effective helminth control strategies for small ruminants in Ethiopia, anthelmintics are exclusively used for the management of the adverse effects of nematodes in sheep. However, the efficacy of anthelmintics is affected by such factors as under dosage, exclusive use of drugs of the same mode of action, substandard quality drugs, and inappropriate use.

Misuse and smuggling of anthelmintics in many forms, such as illegal sales in open markets and irrational administration, is widespread in Ethiopia. In addition, due to the absence of a rational policy for anthelmintic use, methods that can preserve and maintain the efficacy of anthelmintics, and delay or prevent the emergence of anthelmintic resistance are not practiced in any part of the country (10,11).

Albendazole, a broad-spectrum benzimidazole, is the most widely used anthelmintic for the treatment and prevention of nematode infections in sheep in Ethiopia. It is manufactured by many international factories with various trade names, and is imported and distributed by several agents to vast areas in Ethiopia. Furthermore, the recently expanding private veterinary sector in Ethiopia has resulted in a remarkable increase in the number of veterinary

pharmaceutical importers and distributors. Despite the widespread use of albendazole (ABZ) against nematodes in sheep in Ethiopia, there is a paucity of information on the efficacy of benzimidazole anthelmintics (10,11).

For this reason, the present study aimed to evaluate and compare the efficacy of 7 brands of albendazole against gastrointestinal nematodes in naturally infected sheep in Hawassa (southern Ethiopia). In addition, data on the use of anthelmintics for sheep in the study area were collected and compiled using a systematically organized survey. Data on the efficacy of benzimidazoles may help in the design of effective and sustainable control options for helminths in sheep.

## Materials and methods

### Study area

The present study was conducted from April to September 2006 at Hawassa College of Agriculture, Hawassa University, southern Ethiopia. Hawassa is the capital of the Southern Nations, Nationalities, and People's Regional State (SNNPRS), and is located about 275 km south of Addis Ababa, the capital of Ethiopia. Geographically the area lies between lat 4°27' and 8°30' N, and long 34°21' and 39°11' E. Hawassa receives an annual rainfall of 801-1000 mm and during the study period temperature ranged from 20.1 to 25 °C at an average altitude of 1700 m above sea level. The rainy season extends from June to September, with some dry spells in May or June (12). Cattle, sheep, goats, and equines constitute the major livestock in the study area, which are managed under a traditional husbandry system in permanent settlements where crop residues, natural pastures, and some browse species are the main sources of feed. Veterinary services in the study area are generally inadequate.

### Study animals and experimental design

The study included 120 male lambs about 1 year of age that were nearly uniform in size and weight, and were purchased from an open market in the vicinity of Hawassa. The sheep were brought to the

College of Agriculture of Hawassa University. Upon arrival each sheep was checked for infection by parasites, and eggs per gram of feces (EPG) for strongyle-type nematodes was determined on an individual basis. In addition, each sheep was individually marked with a numbered ear tag, and body weight and body condition scores were determined for each sheep, as per the methods of AIGR (13). None of the sheep received any anthelmintic treatment before the start of the study.

After an acclimatization period of 4 weeks the sheep were divided into an untreated control group and 7 treatment groups (15 animals in each group) by blocking, based on EPG of strongyle-type nematodes determined on day 0 (before treatment). Sheep in each group were housed in isolated concrete-floored pens, were fed locally dried hay throughout the study period (to preclude accidental infection by parasites), and were provided with water ad libitum. The animals received Rhodes grass hay as a basal diet supplemented with *Desmodium intortum* hay, and had free access to mineral lick and water. Pretreatment EPG was determined using the McMaster egg counting technique, as described in MAFF (14). The sheep were divided into 8 groups: group 1 served as the untreated control group, and groups 2-8 were treated with ABZ<sub>1</sub>, ABZ<sub>2</sub>, ABZ<sub>3</sub>, ABZ<sub>4</sub>, ABZ<sub>5</sub>, ABZ<sub>6</sub>, and ABZ<sub>7</sub>, respectively. Twelve days post-treatment EPG was determined for each sheep in each group. The efficacy of each brand of albendazole was tested based on both fecal egg count reduction and egg hatch tests. Pooled fecal samples for each group were cultured for larval identification before and after treatment.

#### Questionnaire survey

A questionnaire survey on the current use of anthelmintics in sheep was administered to 100 sheep owners and 7 veterinary drug vendors in the study area. Data on the commonly used anthelmintics, frequency of use, criteria for selection, major source, method of dose determination, and rotation among the classes of anthelmintics were collected and compiled.

#### Brands of albendazole

Sheep in group 1 served as the untreated control group, whereas the sheep in groups 2-8 were treated

with 7 brands of albendazole at the doses recommended by the manufacturers, based on their individual weight. The 7 brands of albendazole used in the study were Alzole (albendazole 300 mg, 3.5 mg kg<sup>-1</sup> of body weight [BW], East African Pharmaceuticals, Addis Ababa, Ethiopia), Albendazole (300 mg, 3.5 mg kg<sup>-1</sup> of BW, Star Laboratories, Lahore, Pakistan), Ashialben (300 mg, 3.5 mg kg<sup>-1</sup> of BW, Ashi Life Science, Mumbai, India), Exiptol (albendazole 300 mg, 3.5 mg kg<sup>-1</sup> of BW, ERFAR S.A., Attiki, Greece), Albenjung (albendazole 300 mg, 3.5 mg kg<sup>-1</sup> of BW, South Korea), Vetaben (albendazole 300 mg, 3.5 mg kg<sup>-1</sup> of BW, Indian Immunologicals, Ltd., Hyderabad, India), and Rangalben (albendazole 300 mg, 3.5 mg kg<sup>-1</sup> of BW, India). All brands were administered orally using calibrated syringes. In this efficacy study the 7 brands of albendazole were randomly designated as ABZ<sub>1</sub>, ABZ<sub>2</sub>, ABZ<sub>3</sub>, ABZ<sub>4</sub>, ABZ<sub>5</sub>, ABZ<sub>6</sub>, and ABZ<sub>7</sub> in order to prevent biasing the results.

#### Fecal collection and examination

Fecal samples were collected from each experimental sheep for coproscopic examination into pre-labeled universal bottles on day 0 (before treatment) and again on post-treatment day 12. Samples were examined for parasite eggs using a saturated salt solution as a flotation fluid within 2 h of collection at the Veterinary Laboratory of Animal and Range Sciences, Hawassa University.

Eggs per gram (EPG) of strongyle-type nematodes was determined for each sample using the modified McMaster technique, according to MAFF (14) and Coles et al. (15), with a sensitivity of 50 eggs g<sup>-1</sup> of feces. Infection by strongyle-type nematodes in each sheep was categorized as light (50-800), moderate (801-1200), and heavy (>1200) based on the EPG value of the pre-treatment fecal samples, as described by Hansen and Perry (16).

#### Efficacy evaluation

##### Fecal egg count reduction test

The efficacy for each brand of albendazole was determined according to the WAAVP recommendations for efficacy evaluation of anthelmintics (15,17). The fecal strongyle egg count reduction percentage (FECR %) was calculated using the following formula:  $FECR\% = 100(1 - X_t/X_c)$ ,

where  $X_t$  and  $X_c$  are the arithmetic mean EPG in the treated (t) and untreated control (c) groups, respectively, on post-treatment day 12. Efficacy was considered poor if the FECRT % was less than 95% or if the lower 95% confidence limit for the reduction was less than 90% (15).

#### Egg hatch test

The egg hatch assay was performed and interpreted as outlined in the WAAVP recommendations (15,17). The technique employed in the present study was similar to that used by Kumsa and Wossene (11). Briefly, pre-treatment samples were pooled for all lambs and undeveloped strongyle eggs from freshly collected feces were recovered using saturated magnesium sulphate as a flotation fluid before processing and within 2 h of collection. Magnesium sulphate from the eggs was removed with excess tap water. The recovered eggs were adjusted to 50-100 eggs in 100  $\mu$ L of distilled water and were incubated for 48 h at 23 °C in serial concentrations of each brand of albendazole dissolved in 1% dimethyl sulphoxide (DMSO; 8 different concentrations for each brand, ranging from 0 to 2.56  $\mu$ L). The control was prepared in 5 replicates, whereas each of the different concentrations was prepared in triplicate. Lugol's iodine was used to stop further hatching, and then all eggs and larvae at each concentration were counted as dead, embryonated, or hatched to  $L_1$  for each brand of albendazole. The percentage of eggs that hatched, embryonated, or died at each concentration was determined by counting the contents of each labeled tube under a microscope. Natural mortality was corrected based on the percentage of eggs that hatched in the controls. An  $LD_{50}$  value for each brand was determined using StatPlus professional statistical software v.3.4.8 for Windows.

#### $L_3$ identification

Approximately 5-g fecal samples from each sheep were pooled for each group and incubated at 27 °C for 7 days, before and after treatment. The  $L_3$  were recovered using the Baermann technique. Then  $L_3$  were counted and identified according to the morphological keys given by MAFF (14) and van Wyk et al. (18). When possible, 100  $L_3$  were differentiated for each group.

#### Data analysis

Descriptive statistics were used to analyze the mean EPG of the helminth egg count, prevalence, and pre-treatment and post-treatment results. Linear correlation was used to analyze differences between body condition scores and EPG counts, and EPG of different body scores were analyzed by pair-wise mean comparisons using SPSS v.15 for Windows (SPSS, Inc., Chicago, IL, USA). The efficacy of the brands of albendazole under evaluation, mean fecal egg counts, percentages of reduction, and 95% upper and lower confidence limits were determined as per the recommendations of Coles et al. (15,17). The chi-square ( $\chi^2$ ) test was used to measure relationships between the prevalence of different genera of parasites and the degree of EPG of strongyle-type nematodes in the experimental sheep. Differences were considered significant at  $P < 0.05$ . Data from egg hatch tests were analyzed to determine the  $LD_{50}$  (the concentration of albendazole required to inhibit 50% of strongyle eggs from hatching).  $LD_{50}$  values of above 0.1  $\mu$ g  $mL^{-1}$  indicated poor efficacy of the evaluated drug (15,17).

## Results

#### Questionnaire survey

The farmers that completed the questionnaire survey indicated that benzimidazoles were the most commonly (87%) used anthelmintic group used in the sheep in the study area (Table 1). The results of the questionnaire survey pertaining to the criteria used for the selection of anthelmintics, sources of anthelmintics, dose determination methods, frequency of anthelmintic treatment, and the farmers' knowledge about annual rotation among the families of anthelmintics are given in Table 1. The interviewed private veterinary drug venders and government veterinary clinics reported that the proportional sale of anthelmintics was as follows: benzimidazoles 81%, imidazothiazoles 17%, and macrocyclic lactones 2%.

#### Pretreatment coproscopic examinations

Coproscopic examination for the presence of parasite eggs in the pretreatment fecal samples revealed 100% strongyle eggs, 15% *Trichuris* spp., and 6.7% *Strongyloides papillosus*, as shown in Table 2.

Table 1. Results of the questionnaire survey on anthelmintic usage for sheep in the study area.

Type of anthelmintic used	Percentage
Benzimidazoles	87
Imidazothiazoles	12
Macrocyclic lactones	1
Anthelmintic selection criteria	
Low price	43
Color	27
Size	11
Veterinarian advice	10
Ease of application	9
Source of anthelmintics	
Open market	42
Government clinics	27
Private pharmacies	20
Illegal sellers	11
Dosage determination method	
Visual estimation	100
Weight of each animal	0
Weight of heaviest animal	0
Frequency of anthelmintic treatment	
Once per year	11
Twice per year	87
Three times per year	2
Greater than 3 times per year	0
Anthelmintic class rotation	
No rotation	100
Rotate annually	0

The prevalence of strongyle-type nematode infection was significantly ( $P < 0.05$ ) higher than the prevalence of infection by other types of gastrointestinal helminths, as indicated in Table 2. The greatest proportion of infected sheep had heavy infection (86.6%) by strongyle-type nematodes, versus moderate (4.2%) and light (9.2%) infection, as presented in the Figure. The results of the present study show that there was an inverse linear relationship between both individual and mean EPG in pre-treatment fecal samples, and body condition score in naturally infected sheep.

Efficacy evaluation

Efficacy evaluation on post-treatment day 12 revealed variable reductions in fecal strongyle egg

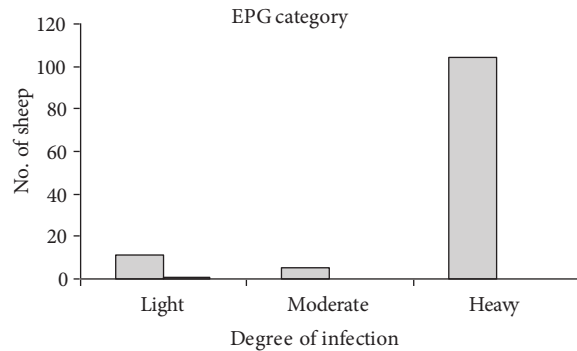


Figure. Degree of infection of study sheep based on EPG of pre-treatment fecal samples.

Table 2. Prevalence and mean EPG of nematodes from pre-treatment samples of the experimental sheep.

Group	<i>Strongyle type egg</i>		<i>Trichuris spp.</i>		<i>S. papillosus</i>	
	P	EPG	P	EPG	P	EPG
Control (n = 15)	100	4263	2	200	0.7	100
ABZ <sub>1</sub> (n = 15)	100	3920	1.8	150	0.8	200
ABZ <sub>2</sub> (n = 15)	100	3615	2	100	0.7	150
ABZ <sub>3</sub> (n = 15)	100	4010	1.6	200	0.8	100
ABZ <sub>4</sub> (n = 15)	100	5000	2	100	0.6	300
ABZ <sub>5</sub> (n = 15)	100	4000	1.8	50	0.7	200
ABZ <sub>6</sub> (n = 15)	100	4040	2	100	0.8	150
ABZ <sub>7</sub> (n = 15)	100	3450	1.8	150	0.7	200
Total (n = 120)	100	2114	15	131	5.8	175

P: prevalence; EPG: average eggs per gram of feces; n: number of sheep in each group.



counts in the sheep treated with 7 brands of albendazole. High-level reductions in fecal egg counts were observed in the sheep treated with ABZ<sub>1</sub>, ABZ<sub>2</sub>, ABZ<sub>4</sub>, ABZ<sub>5</sub>, and ABZ<sub>7</sub> whereas a low-level of efficacy was recorded in the sheep treated with ABZ<sub>3</sub> and ABZ<sub>6</sub>, as shown in Table 3. FECRT % values of 99%, 100%, 95%, 100%, 99%, 95%, and 97%, and LD<sub>50</sub> values of 0.06, 0.05, 0.11, 0.05, 0.07, 0.12, and 0.08 were recorded for ABZ<sub>1</sub>, ABZ<sub>2</sub>, ABZ<sub>3</sub>, ABZ<sub>4</sub>, ABZ<sub>5</sub>, ABZ<sub>6</sub>, and ABZ<sub>7</sub>, respectively (Table 3). These findings suggest agreement between the LD<sub>50</sub> values obtained in the egg hatch tests and the FECRT % results, as both efficacy studies showed variation in the efficacy of the tested brands of albendazole.

### L<sub>3</sub> identification

Identification of L<sub>3</sub> from pretreatment coproculture samples revealed a significantly (P < 0.05) higher proportion of *Haemonchus* spp. than other gastrointestinal nematodes in all the study groups, as shown in Table 4. In the treatment groups only larvae of *Haemonchus* spp. were identified in post-treatment coproculture fecal samples.

### Discussion

The questionnaire survey revealed that farmers in the study area were engaged in several practices that limit the efficacy of anthelmintics. In addition, the

Table 3. FECRT% and post-treatment EPG of strongyle type nematodes of the experimental sheep.

Group	Post-EPG	FECRT (%)	UCL	LCL	Efficacy status	LD <sub>50</sub>
Control (n = 15)	8025	-	-	-	-	0.04
ABZ <sub>1</sub> (n = 15)	70	99	100	95	High	0.06
ABZ <sub>2</sub> (n = 15)	40	100	100	97	High	0.05
ABZ <sub>3</sub> (n = 15)	380	95	98	88*	Low	0.11
ABZ <sub>4</sub> (n = 15)	15	100	100	99	High	0.05
ABZ <sub>5</sub> (n = 15)	60	99	100	97	High	0.07
ABZ <sub>6</sub> (n = 15)	365	95	98	89*	Low	0.12
ABZ <sub>7</sub> (n = 15)	205	97	99	93	High	0.08

Post-EPG, post-treatment eggs per gram of feces; FECRT, fecal egg count reduction test; UCL; upper confidence limit; LCL; lower confidence limit; \* indicates LCL less than 90%; n: number of sheep in each group.

Table 4. Percentages of third stage nematode larvae identified from pre-treatment coprocultures of the study sheep.

Group	Genera of nematodes in percentage						
	Haem	Oeso	Trich	Telado	Buno	Chab	<i>S. papillosus</i>
Control (n = 15)	60*	13	17	5	2.1	1.7	1.2
ABZ <sub>1</sub> (n = 15)	54.5*	14	18	7.2	3	2.9	0.4
ABZ <sub>2</sub> (n = 15)	56*	12.8	15	7	4.3	3.2	1.7
ABZ <sub>3</sub> (n = 15)	58*	14	14	5.2	4	4	0.8
ABZ <sub>4</sub> (n = 15)	59*	13.6	13	7.1	2.9	3.1	1.3
ABZ <sub>5</sub> (n = 15)	57*	10	18	6.4	3.6	3	2.1
ABZ <sub>6</sub> (n = 15)	52*	16	13.9	8.3	4.1	3.7	2
ABZ <sub>7</sub> (n = 15)	61*	17	13	4.7	1.3	2.1	0.9

Haem = *Haemonchus* spp.; Oeso = *Oesophagostomum* spp.; Trich = *Trichostrongylus* spp.; Telado = *Teladorsagia* spp.; Chab = *Chabertia* spp.; *S. papillosus* = *Strongyloides papillosus*; n: number of sheep in each group; \* indicates significantly higher proportion.

study results show that benzimidazoles were the most widely used group of anthelmintics, followed by imidazothiazoles and macrocyclic lactones. These findings are in agreement with those reported by Arece et al. (19).

The significantly higher prevalence of strongyle-type nematodes than *Trichuris* and *S. papillosus* we observed in the pre-treatment fecal samples was most probably due to the fact that the study included relatively young and poorly managed sheep bought from farmers in the study area. Furthermore, prior to the FECRT investigation the experimental sheep were managed under a system with a high stocking rate, inadequate nutrition, and poor veterinary services typical of smallholder farms in southern Ethiopia, which also explains why the majority of the study sheep had a heavy degree EPG of strongyle-type nematodes. These results are similar to those reported by Kumsa and Wossene (7), Regassa et al. (20), Sissay et al. (21), and Thomas et al. (8).

The inverse relationship we observed between the high EPG values for pre-treatment fecal samples and body condition score in the study sheep with naturally acquired infection indicates that gastrointestinal nematodes caused significantly negative effects on growth and productivity in growing young ruminants, which is similar to the results published by Githigia et al. (22).

Studies on the efficacy of anthelmintic drugs are useful for establishing and maintaining effective sustainable control strategies against helminths, especially in small ruminants. The efficacy evaluation of the 7 brands of albendazole carried out in the present study and interpreted as per the WAAVP recommendations showed variations in efficacy among the brands. The results of this study based on FECRT and egg hatch test methods show similar variations in efficacy of the evaluated brands of albendazole. The egg hatch test is considered a more sensitive technique than FECRT, which validated and supported this assertion. Using both tests, ABZ<sub>3</sub> and ABZ<sub>6</sub> were observed to be less effective against nematodes in the study sheep than the other brands. We think the most likely explanation for the variation in efficacy obtained in the present study is the low quality of the brands, rather than the administered

doses of the tested drugs, which is in agreement with previous studies by van Wyk et al. (23), Hussein et al. (10), Keyyu et al. (24), and Ancheta et al. (25). The low efficacy of some brands of albendazole could have negative effects on the health and productivity of animals, and could possibly be one of the factors in the emergence of anthelmintic resistance in nematodes in small ruminants in the study, as reported by Arece et al. (19) and Ram et al. (26).

The observed predominance of L<sub>3</sub> of *Haemonchus* spp. in the pre-treatment coprocultures is consistent with the earlier work by Kumsa and Wossene (7), and Sissay et al. (21). Due to great ecological and biological plasticity, *Haemonchus* spp. were the only L<sub>3</sub> identified from the coproculture samples after treatment with less efficacious brands, supporting many previous studies that reported the association between *Haemonchus* spp. and reduced efficacy of anthelmintics (27-30).

In conclusion, the results of the present study show that gastrointestinal nematodes in sheep in the study area are susceptible to 5 brands of albendazole. On the other hand, 2 brands of albendazole were less efficacious against nematodes in the study sheep. Hence, standardized and reliable quality control methods that ensure the efficacy of anthelmintics based on quantitative analysis of active ingredients, purity, disintegration, and bioavailability should be implemented by the registration authorities in Ethiopia. In addition, so as to maintain and prolong the lifespan of the efficacy of available anthelmintics farmers should be educated by veterinary extension programs about the importance of rational anthelmintic use, such as the correct dose, annual rotation among anthelmintic groups, and avoiding the risk factors that lead to reduced efficacy and anthelmintic resistance. Hence, farmers and veterinary professionals should consider the poor efficacy of anthelmintics as a serious problem and the routine diagnosis of infections by helminths should be complemented by standardized efficacy evaluation techniques. Further studies, however, are needed to assess the efficacy status of commonly used front-line anthelmintics in different agroecologies, animal species, and management systems, with a particular focus on the associated economic impact of the problem.



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