

Full Length Research Paper

Prevalence of internal parasites in sheep/goats and effective economic de-worming plan at upland Balochistan, Pakistan

Abdul Razzaq¹, Muhammad Islam², Sarfraz Ahmad¹, Kamel Shideed³, Farouk Shomo³ and Mohammad Athar^{4,5*}

¹Arid Zone Research Center, Brewery Road, Quetta, Pakistan.

²ICARDA, Arid Zone Research Center, Brewery Road, Quetta, Pakistan.

³International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

⁴California Department of Food and Agriculture, Pest Detection and Emergency Projects, 3288 Meadowview Road, Sacramento, CA 95832, USA.

⁵Department of Food Science and Technology, University of Karachi, Karachi-75270, Pakistan.

Accepted 14 March, 2011

Experiments were conducted on sheep and goats during 2006 to 2007 to investigate the prevalence, control and efficacy of anthelmintics against internal parasites at two sites (Loralai and Qila Saifullah) in Balochistan, Pakistan. Faecal samples were collected directly from the rectum of 10 to 20% animals from each flock before the administration of anthelmintics. About 87 to 93% sheep/goats were infested with 10 types of internal parasites. These were *Dictyocaulus*, *Moniezia benideni*, *Moniezia expansa*, *Fasciola hepatica*, *Strongyloides*, *Nematodirus*, *Trichostrongylus*, *Cooperia*, *Trichuris ovis* and *Bunostomum*. The level of parasitic infestation was mostly between 500 to 2000 eggs per gram (EPG) of faeces. Prior to execution of experiment, more than 80% animals showed high parasitic infectivity (>1000 EPG). Drenching of different anthelmintics such as Nilzan, Ivermectin, Zodec, Bendex, Oxadec and Albensil in infested animals with different durations lowered the internal parasitic level (300 EPG) and also eliminated most of the internal parasites except *Dictyocaulus*. Albensil was found more effective and economical against internal parasites of sheep and goats. It is concluded that 3 to 4 months interval de-worming with suitable anthelmintics enhance the small ruminants' productivity.

Key words: Small ruminants, internal parasites, anthelmintics and economic parasitic control.

INTRODUCTION

Livestock sub-sector accounts for 49.1% of agriculture value added and 11.4% of National Gross Domestic Product. There are 26.49 million sheep and 53.79 million goats in the country and 12.8 million (48%) and 11.78 million (22%) of these, respectively are raised in Balochistan province of Pakistan (Agricultural Census Organization, 2006).

Animal production systems in Balochistan traditionally fall into three categories: Nomadic (30%), transhumant

(60%) and sedentary (10%) (Buzdar et al., 1989). In Balochistan, a number of constraints in rearing livestock exist and badly impair animal production operations and thus, affect the gross domestic product and also the socio-economic status of livestock farmers (Islam et al., 2004; Mirza et al., 2009; Mohammad and Atiq-ur-Rehman, 2000).

Internal parasites pose one of the major health limitations to grazing animals throughout the world. Sheep and goats are more susceptible to internal parasites than other livestock, due to their grazing behavior and poor immunity (Durrani et al., 1981). The impact of parasitic diseases varies greatly between countries and between regions, depending on climate

*Corresponding author. E-mail: atariq@cdfa.ca.gov. Tel: 916-262-0855. FAX: 916-262-2059.

and the intensification of farming in the area (Radostits et al., 1994). Khan et al. (1988a, b) reported that 100% sheep in upland Balochistan get infected with internal parasites. Razzaq et al. (2002) recorded overall internal parasitic infestation in 93% sheep and 80% goats at Asghara valley in district Ziarat.

The present study was designed to monitor and assess its control strategies of the incidence of different internal parasites in sheep and goats farmers flock at two districts, Loralai Nali walizai and Qila Saifullah (Alozai) of upland Balochistan.

MATERIALS AND METHODS

Description of Loralai district

The Loralai district lies between 30°, 51' to 31°, 58' N latitudes and 67°, 27' to 69°, 34' E longitudes. At the lower elevation, especially in the south and East, temperature is more uniform but hot in summer.

Description of Qila Saifullah district

Qila Saifullah district is located at 30°-51° to 31°-70° north latitudes and from 67°-27° to 69°-34° east longitudes.

Experiments (animals, site and duration)

Three experiments were conducted on prevalence, control and efficacy of anthelmintics against sheep and goats internal parasites.

Experiment one: District Qila Saifullah

The first experiment was conducted at Alozai (District Qila Saifullah) on three farmers sheep flocks (A, B and C comprising of 50, 43 and 50 animals, respectively) of Shinwar and Balochi breed during September 2006 to March 2007. Two anthelmintics, Nilzan (Levamisole HCl + Oxytoclozanide) 1 ml/5 kg body weight and Ivermectin 1 ml/50 kg body weights were administered to sheep in group A and B, respectively with three months intervals (during September 2006, December 2006 and March 2007) and group C was kept as a control to monitor the internal parasitic load.

Experiment two: District Loralai

The second experiment was conducted on three sheep flocks and four goat flocks comprising 49 sheep and 44 goats selected from Nali WaliZai (District Loralai) during September 2006 to March 2007. These animals were administered Zorox Gold (oxfendazole + oxytoclozanide + cobalt + selenium) 1 ml/10 kg body weight during September 2006 and repeated during December 2006 for the control of internal parasites.

Experiment three: Monitoring the worm load

In the third experiment, 20 farmers from two sites, Qila Saifullah and Loralai, were used to monitor the worm load in sheep and goats from August to December 2007 and divided into five groups to check the efficacy of five anthelmintics, Nilzan ((Levamisole HCl +

Oxytoclozanide) 1 ml/ 5 kg body weight, Bendex (albendazol + cobalt + selenium) 1 ml/ 5 kg body weight, oxadec (oxfendazole + cobalt + selenium) 1 ml/ 5 kg body weight, albensil (Albendazol) 1 ml/10 kg body weight and Ivermectin 1 ml/50 kg body weight available in the local market. These anthelmintics were administered to sheep and goats and effectiveness against the internal parasites evaluated with an interval of four months.

All these farmer flocks were raised on daily (6 to 8 h) range grazing, round the year and minimum (200 to 300 g/head) supplemental feeding (barley grain, wheat, maize grain) offered to adult breeding stock during winter/breeding cycle (November to February).

Analysis of faecal samples for incidence of internal parasites

Almost all the sheep were found apparently healthy and only three showed bottle jaw and few with diarrhea, coughing and rough wool at pre medication. The parasitic prevalence was identified on the basis of faecal samples analysis. Faecal samples from these experimental sheep were analyzed microscopically using direct slide method for the presence of parasitic ova or larvae. Further identification was made with the help of micrometry like shape, color, size and other characteristics of ova/larvae as described by Thienpont et al. (1979). In addition, parasitic egg per gram (EPG) of faeces was also counted by McMaster technique as described by Urquhart et al. (1996).

Statistical analysis

Data regarding the prevalence of internal parasites, pre- and post-medication were analyzed with respect to percentage of infectivity, mean and standard error of EPG by computer Microsoft Excel program.

RESULTS

Prevalence of sheep internal parasites and its control at Alozai (District Qila Saifullah)

The experiments were conducted to assess the prevalence and control of internal parasites of sheep at Alozai (District Qila Saifullah). Microscopic examination of these faecal samples showed that, prior the administration of anthelmintics, 87 to 93% sheep were infested with 10 types of internal parasites (Table 1).

The quantitative faecal examination prior to medication (September 2006) revealed that, infestation of five parasites (*Dictyocaulus*, *Moniezia benideni*, *Moniezia expansa*, *Strongyloides*, *Nematodirus*) was higher (EPG 1200 ± 44.7 to 2000 ± 0.0) and the remaining parasites (*Fasciola hepatica*, *Trichostrongylus*, *Cooperia*, *Trichuris ovis* and *Bunostomum*) showed lower infestation (200 ± 0.0 to 952.6 ± 90.6). While post medication sheep (up to seven months) in groups A and B were found free of these parasites except *Dictyocaulus* but with lower EPG (300 ± 0 and 300 ± 57.7).

The results indicated that, sheep in control group (C) had higher (87 to 100%) internal parasitic infestation

Table 1. Incidence of internal parasites in sheep treated with Ivermectin and Nilzan compared with control at Aloe Zai.

Internal parasite Group	Sep-06*			Sep-06*			Dec 06*			Mar 07*		
	A	B	C	A	B	C	A	B	C	A	B	C
No. of animals	50	43	50	50	43	50	50	50	43	50	43	50
Animals sampled	15	15	15	15	15	15	15	15	15	15	15	15
<i>Dictyocaulus</i>	6	5	6	2	3	5	2	3	6	3	-	8
<i>M. benideni</i>	2	3	1	-	-	1	1	-	-	-	-	1
<i>M. expensa</i>	-	1	-	-	-	2	-	4	4	-	-	-
<i>F. hepatica</i>	8	5	6	-	-	8	-	-	3	-	-	-
<i>Strongyloides</i>	2	3	1	-	-	-	-	1	1	-	-	2
<i>Nematodirus</i>	1	0	1	-	-	2	2	1	2	2	-	2
<i>Trichostrongylus</i>	-	2	3	-	-	2	-	-	1	-	1	2
<i>Cooperia</i>	-	1	2	-	-	2	-	-	1	-	-	-
<i>T. ovis</i>	2	2	-	-	-	1	2	5	4	2	1	8
<i>Bunostomum</i>	1	6	1	-	1	4	-	2	3	2	1	1
No. of animal infested	14	13	14	2	3	15	3	5	13	4	2	13
Infestation %	93	87	93	13	12	100	20	33	87	27	13	87

Table 2. Incidence and control of internal parasites of sheep treated with Ivermectin and Nilzan at Aloe Zai.

Parasite	Before treat (143 sheep) September		After treat (143 sheep) Avg of Dec- March					
	Infected (%)	Mean EPG ± SE	Infected (%)			Mean EPG ± SE		
			A	B	C	A	B	C
			50	43	50	50	43	50
<i>Dictyocaulus</i>	38.7	1294.1 ± 36.8	4.4	6.6	11.1	300 ± 0	300 ± 57.7	1200 ± 209.7
<i>M. benideni</i>	13.3	1200.2 ± 44.7	-	-	2.2	-	-	1000 ± 0.0
<i>M. expensa</i>	2.2	1400.4 ± 00.0	-	-	4.4	-	-	1133 ± 0.0
<i>F. hepatica</i>	42.2	952.6 ± 90.6	-	-	4.4	-	-	1000 ± 0.0
<i>Strongyloides</i>	13.3	1400.2 ± 36.5	-	-	-	-	-	-
<i>Nematodirus</i>	4.4	2000.5 ± 00.0	-	-	4.4	-	-	1700 ± 0.0
<i>Trichostrongylus</i>	11.1	200.2 ± 00.0	-	-	4.4	-	-	350 ± 0.0
<i>Cooperia</i>	6.6	300.2 ± 57.7	-	-	4.4	-	-	350 ± 0.0
<i>T. ovis</i>	8.8	400 .4 ± 40.8	-	-	2.2	-	-	400 ± 0.0
<i>Bunostomum</i>	17.7	300.2 ± 32.7	-	-	4.4	-	-	320 ± 40.8

SE, Standard error; EPG, eggs per gram of faeces; N, total number of animals.

throughout the study and it remained low (13 to 33%) in de-wormed sheep of groups A and B.

Prevalence of internal parasites in sheep and goats and its control at Nali walizai (District Loralai)

A total of 44 sheep and 49 goats were selected from this site. Microscopic examination of faecal samples showed that, prior to anthelmintics (during September 2006), 87% of sheep and 67% of goats were infested with nine types of internal parasites (Table 2). The quantitative faecal examination showed higher EPG (1500 ± 75 and 2000 ± 91.2) of two parasites *T. ovis* and *Nematodirus* in sheep (Table 4). Almost all the goats showed higher EPG (>

1000) with all recorded parasites except *Dictyocaulus* and *Bunostomum* (< 1000 EPG). These results showed that, animals treated with broad-spectrum anthelmintics reduced internal parasitic level from 87% to minimum 6%.

Efficacy and economic analysis of locally available anthelmintics against internal parasites of sheep and goats

The experiments showed that, almost 60 to 80% sheep and goats in the study area were infested with ten different internal parasites (Table 5). In this study, the economical analysis of anthelmintics based on efficacy and cost showed that, Albensil was found highly effective

Table 3. Incidence of internal parasites in sheep and goats treated with Zorox gold.

Internal parasite Group	Sep-06*		Sep-06**		Dec-06*		Mar-07*	
	Sheep	Goat	Sheep	Goat	Sheep	Goat	Sheep	Goat
No of animal	49	44	49	44	49	44	49	44
Animals sampled	15	15	15	15	15	15	15	15
<i>Dictyocaulus</i>	7	6	2	1	3	2	4	6
<i>M. benideni</i>	0	1	0	0	0	0	0	0
<i>M. expensa</i>	0	4	0	0	0	0	0	0
<i>F. hepatica</i>	5	0	0	0	0	0	0	0
<i>Strongyloides</i>	0	1	0	0	0	0	0	0
<i>Nematodirus</i>	4	1	0	0	0	0	0	0
<i>Trichostrongylus</i>	0	1	0	0	0	0	0	0
<i>T. ovis</i>	4	6	0	0	1	0	0	0
<i>Bunostomum</i>	2	4	0	0	0	0	0	0
No of animals	13	10	2	1	3	2	4	6
Infestation %	87	67	13	6	20	13	27	40

*Before treatment and ** seven days after the treatment.

and economically followed by Oxadec, Nilzan, Bendex and Ivermectin (Table 6).

DISCUSSION

Internal parasites pose one of the major health limitations for grazing animals and cause considerable economic loss throughout the world (McLeod, 1995; Waller, 2004). Although, there are numerous internal parasites, only a few of them account for the majority of problems for grazing livestock. The results of this study revealed that, parasitic infection is common in sheep and goat. The infection pattern (infection level and intensity) in Balochistan are primarily affected by parasite contamination, environmental conditions and management practices. In the present study, overall incidences of internal parasites before treatment at both research sites were recorded higher (up to 100 %) in sheep than in goats (67%). These results coincide with Khan et al. (1988a, b) and Razzaq et al. (2002) who observed internal parasitic infestation of 93% in sheep and 80% in goats.

The present study revealed infestation of 10 types of internal parasites (*Dictyocaulus*, *M. benideni*, *M. expensa*, *F. hepatica*, *Strongyloides*, *Nematodirus*, *Trichostrongylus*, *Cooperia*, *T. ovis* and *Bunostomum*) in sheep and goats. Khan et al. (1988b) reported same parasites along with other three species of parasites (*Haemonchus contortus*, *Marshallagia marshalli*, and *Avitellina centripulata* (13%)) prevalent in sheep in Kovak valley (Kalat district) of Balochistan. Khan et al. (1988a) also observed other internal parasites in sheep in upland districts of Balochistan which were *M. marshalli*, *Oesophagostomum*, *H. contortus*, *Cotylophoron*,

Paramphistomum cervi and *A. centripulata*. The internal parasitic incidences reported by Khan et al. (1988a, 1988b) were higher than what was observed in the present study. Difference in the incidence of different internal parasitic species in the present and other studies conducted in different locations of Balochistan may be due to different ecologies, temperature, forage and fodder availability. Durrani et al. (1981) indicated that, incidence of few species of internal parasites was relatively higher and lower under the arid conditions of upland Balochistan when compared with semi-humid, subtropical climate of Punjab. Radostits et al. (1994) mentioned that warm and wet weather provide suitable conditions for the translation of eggs to larvae in the majority of helminths. The areas having severe winter and dry summer reduced the parasitic burden on the local livestock as seen in the present study (80 to 90%).

Resistance of *Dictyocaulus*, recorded in present study is now reported worldwide (Besier and Love, 2003; Kaplan et al; 2004). Zajac and Gipson (2000) mentioned that, these parasites are difficult to manage because they have developed resistance to all available commercial de-wormers on some farms. However, the recommended dose of almost all the anthelmintic has shown positive results in sheep and goats in this study.

It is found that the incidences of internal parasites can be reduced up to 6 to 33% by using broad-spectrum anthelmintics like Nilzan (Levamisole and Oxyclozanide), Zorox gold (Oxyclozanide, Oxfendazole selenium and cobalt) and Ivectin (Ivermectin 1%). Our results show that, Ivectin was found 100% effective against internal parasites of sheep and goats except *Dictyocaulus*, which might be due to the development of resistance. Han-Bo et al. (1997) reported Ivermectin 100% effectiveness against internal and external parasites.

Table 4. Incidence of internal parasites in sheep and goat treated with Ivermectin and Nilzan compared with control at Nali WaliZai.

Parasite	Before treatment				After Treatment			
	Sheep (49)		Goat (44)		Sheep (49)		Goat (44)	
	Infected (%)	Avg.EPG±SE	Infected (%)	Avg. EPG ± SE	Infected (%)	Avg. EPG ± SE	Infected (%)	Avg. EPG ± SE
<i>Dictyocaulus</i>	46.66	800 ± 15	6	616.6 ± 47.7	2	1000 ± 0	6.66	2000 ± 0
<i>M.benideni</i>	-	-	1	1400 ± 0	-	-	-	-
<i>M. expensa</i>	-	-	4	1150 ± 64.5	-	-	-	-
<i>F.hepatica</i>	33.33	600 ± 54.7	-	-	-	-	-	-
<i>Strongyloides</i>	-	-	6.66	1000 ± 0	-	-	-	-
<i>Nematodirus</i>	26.66	2000 ± 91.2	6.66	1000 ± 0	-	-	-	-
<i>Trichostrongylus</i>	-	-	6.66	2000 ± 0	-	-	-	-
<i>T.ovis</i>	26.66	1500 ± 75	40	1200 ± 276.8	-	-	-	-
<i>Bunostomum</i>	13.33	500 ± 0	26.66	325 ± 75	-	-	-	-

Table 5. Internal parasitic infestation in sheep/goats and efficacy of different anthelmintics against parasites.

Animal sampled	Before treat (August 2007)			Prevalence (%) after Treatment (September to December 2007)				
	No of Inf	No of Prev	EPG	Nilzan	Bendex	Oxadec	Albensil	Ivermectin
	50			10	10	10	10	10
<i>Dictyocaulus</i>	13	26	1307.69 ± 32.93	-	2(800 ± 0)	-	-	2(1133 ± 296)
<i>M.benideni</i>	4	8	1600 ± 91.28	-	-	-	-	-
<i>M. expensa</i>	2	4	1400 ± 0	-	-	-	-	-
<i>F.hepatica</i>	9	18	1744.44 ± 202.1	-	-	-	-	-
<i>Strongyloides</i>	7	14	1214 ± 76.93	-	-	-	-	-
<i>Nematodirus</i>	1	2	1600 ± 0	-	-	-	-	-
<i>Bunostomum</i>	1	2	4000 ± 0	-	-	-	-	-
Total no. of infested	28	56%		-	10%	-	-	10%
No of animals	98			18	22	17	20	21

Egg counts in excess of 1000 are generally considered indicative of heavy infection and those over 500 of moderate infection (Urquhart et al., 1996). In the present study, 80% infected animals showed high EPG (>1000) and 20% showed low EPG (< 500). The high parasitic infestation in the

animals of the study area may be due to heavy stocking rates and insufficient pasture rest periods which contribute continuous transmission of parasitic diseases in sheep and goats.

The prevalence of parasites in Balochistan may be due to lack of de-worming and other health

practices and feed management on regular basis to prevent parasitic attack on animals. It was observed in this study that, most of the flocks were sedentary and they were under strict confinement which leads to high risk of helminth infection (Anene et al., 1994).

Table 6. Economic analysis of anthelmintics against internal parasitic infestation.

Name of medicine	Nilzan	Bendex	Oxadec	Albensil	Ivermectin
Dose rate (LBW)	1 ml /5 kg	1 ml /5 kg	1 ml /5 kg	1 to 2 ml /20 kg	1 ml /50 kg
Price per dose (PRs.)	4.86	3.60	4.00	2.40	4.00
Grading for effectiveness	3	4	2	1	5

One US \$ = 80 PRs.

It may be mentioned that, in Balochistan, all the animals are kept together day and night. The diseased and the healthy animals utilize same watering source and range grazing. Under this situation, it is difficult to completely eradicate the internal parasite infestation. In addition, round the year grazing on same range-lands leads to re-infestation of internal parasites. It would be imperative to administer available anthelmintics in the market in the spring and autumn properly, as small ruminants of Balochistan have not developed resistance against anthelmintics and it will show better results to improve the livelihood of local people.

ACKNOWLEDGEMENTS

The authors are grateful and wish to acknowledge the financial and technical support of AZRC (Arid Zone Research Center), ICARDA (International Center for the Agriculture Research in the Dry Areas) and FAO Project GCP/PAK/095/USA, entitled, "Food Security/Poverty Alleviation in Arid Agriculture-Balochistan, Pakistan," funded by USAID for the completion of this study.

REFERENCES

- Agricultural Census Organization. (2006): Livestock Census (2006). Balochistan. Agricultural Census Organization, Government of Pakistan, Lahore.
- Anene BM, Onyekwodiri EO, Chime AB, Anika SM (1994). Gastrointestinal parasites in sheep and goats of Southern Nigeria. *Small Rumin. Res.* 13: 187-192.
- Besier RB, Love SCJ (2003). Anthelmintic resistance in sheep nematodes in Australia: the need for new approaches. *Aust. J. Exp. Agric.* 43: 1383-1391.
- Buzdar N, Nagy JG, Sabir GF, Keatinge JDH (1989). Animal Raising in Highland Balochistan: A Socio-economic Prospective. MART/ AZRI project. Arid Zone Research Institute, Brewery Road Quetta.
- Durrani MS, Chaudhry NI, Anwar AH (1981). The incidence of gastrointestinal parasites in sheep and goats of Jhelum Valley (Azad Jammu Kashmir). *Pak. Vet. J.* 1: 164-165.
- Han-Bo M, Cheng L, XiangWJ, Ning WJ, Sheng ZJ, An YC, Han B, Ma L, Wang J, Wu J, Zhou J, Yin Y (1997). Removal of sheep parasites using Ivermectin. *Chinese J. Vet. Med.* 23: 5-6.
- Islam M, Ahmad S, Afzal M (2004). Drought in Balochistan, Pakistan: Prospects and Management. Yak Production in Central Asian Highlands, International Livestock Research Institute, Nairobi, Kenya.
- Kaplan RM, Burke, JM, Terrill TH, Miller JE, Getz WR, Mobini S, Valencia E, Williams MJ, Williamson LH, Larsen M, Vatta AF (2004). Validation of the FAMACHA® eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. *Vet. Parasitol.* 123: 105-120.
- Khan KNM, Rehman A, Chaudhry MBA (1988a). Incidence of internal and external parasites in sheep in Kovak Valley (Kalat District) upland Balochistan. Research Report No.13, The MART/AZR Projects ICARDA-PARC AZRI, Quetta, Balochistan.
- Khan KNM, Rehman A, Munir M, Khan BR (1988b). Incidence of internal parasites of sheep in upland districts of Balochistan. Research Report No. 18, The MART/AZR Projects ICARDA-PARC, AZRI Quetta, Balochistan.
- McLeod RS (1995). Costs of major parasites to the Australian livestock industries. *Int. J. Parasitol.* 25: 1363-1367.
- Mirza SN, Athar M, Qayyum M (2009). Effect of drought on rangeland productivity and animal performance in dryland region of Balochistan, Pak. *Agric. Consp. Sci.* 74: 105-109.
- Mohammad F, Atiq-ur-Rehman (2000). Livestock and Rangelands. Background Paper for Balochistan Conservation Strategy. IUCN, Quetta.
- Radostits OM, Blood DC, Gay CC (1994). *Veterinary Medicine. A textbook of diseases of cattle, sheep, pig, goat and horses.* 7th edition. Bath Press, Avon, Great Britain. p. 1367.
- Razzaq A, Rafique S, Tareen S (2002). Incidence of internal parasites in sheep and goat at Asghara valley, District Ziarat, Balochistan. *J. Agric. Sci.* 3: 43-50.
- Thienpont D, Rochette F, Vanparijs OFV (1979). Diagnosing helminthiasis through carpological examination. Janssen Research Foundation, Beerse Belgium.
- Urquhart GM, Armour J, Duncan JL, Jennings FW (1996). *Veterinary Parasitology.* 2nd edition. The Faculty of Veterinary Medicine. The University of Glasgow, Scotland.
- Waller PJ (2004). Management and control of nematode parasites of small ruminants in the face of total anthelmintic failure. *Trop. Biomed.* 21: 7-13.
- Zajac A, Gipson T (2000): Multiple anthelmintic resistances in goat herd. *Vet. Parasitolol.* 87: 163-172.