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SEASONAL EGG OUTPUT OF GASTRO-INTESTINAL PARASITES IN WILD UNGULATES IN A MEDITERRANEAN AREA (CENTRAL ITALY)

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ABSTRACT - Seasonal egg (or oocyst) output of gastro-intestinal parasites of wild ungulates was studied in a Mediterranean protected area, the Monti Livornesi Park (Livorno, Tuscany region, Central Italy). Samples of faeces of wild boars (*Sus scrofa*) and mouflons (*Ovis ammon*) were collected monthly for one year. The observed trends of egg output were analysed taking into account seasonal variations of temperature and rainfall, life-cycle and survival strategy of parasites, and health condition of hosts. In our Mediterranean study area, the peaks of egg output appear in different months according to different biology and survival strategies of parasites.

Key words: wild boar, mouflon, parasites eggs output, seasonality, Italy

RIASSUNTO - ***Emissione stagionale di uova di parassiti gastrointestinali in cinghiali (*Sus scrofa*) e in mufloni (*Ovis ammon*) di un'area mediterranea (Italia centrale)***. E' stata studiata l'emissione stagionale di uova (o oocisti) di parassiti nelle feci di ungulati selvatici in una zona mediterranea protetta, il Parco dei Monti Livornesi (Livorno, Toscana, Italia Centrale). Per un anno sono stati raccolti mensilmente campioni di feci di Cinghiale (*Sus scrofa*) e di Muflone (*Ovis ammon*). Gli andamenti osservati di emissione di uova sono stati analizzati tenendo conto delle variazioni di temperatura e piovosità stagionali, del ciclo biologico e della strategia di sopravvivenza dei parassiti, e delle condizioni sanitarie dell'ospite. E' risultato che in una zona mediterranea come quella considerata i picchi di emissione di uova appaiono in mesi differenti in relazione alla biologia e alle strategie di sopravvivenza dei diversi parassiti.

Parole chiave: cinghiale, muflone, emissione di uova di parassiti, stagionalità, Italia

INTRODUCTION

The problem of evaluating the impact of macroparasites on the health conditions of wild animals and on the contamination of the environment is of ecological importance and increasing economic relevance. The study of the

host–parasite–environment relationship is a complex problem because of many interacting factors, such as climatic conditions, habitat pollution, health condition of hosts, survival strategy of parasites, density dependent factors and human interference (Anderson and May, 1978; Genchi *et al*., 1991 and 1993; Hudson and Dobson, 1995; Stancampiano *et al*., 2001).

In this general frame, one aspect that can be focused on is the seasonality of gastro–intestinal parasite egg output of wild animals. The presence of predictable peaks or trends in egg dropping is linked to the possibility of planning effective animal management actions, in order to reduce contamination of the soil and diffusion of parasites, and to improve the health condition of wild animals. (Larsen and Roepstorff, 1999; Kraglund *et al*., 2001). Furthermore, the improved knowledge concerning these natural phenomena may be transferred to parasite control in domestic animals (Stancampiano, 2004).

Seasonality in egg output shows different features if the area is characterized by relevant climatic variations or by mild homogeneous climate such as in the Mediterranean zone. While in the first case parasite larval recruitment is clustered in time, in the second case this trend should be smoothed down (Guberti *et al*., 2000; Stancampiano *et al*., 2001). In addition, other factors influence the egg output, mainly the health status of the hosts and the life-cycle of the parasites.

The problem of seasonal egg output trends in livestock has been widely treated in literature. The corresponding problem in the case of wild animals has been considered less frequently whether in Italy (e.g. Genchi *et al*., 1982, 1990 and 2000; Lanfranchi and Rossi, 1988; Poglayen *et al*., 1996 and 2002) or in other countries (e.g. Frechette, 1978; Rahman and Collins, 1990; Muller-Graf *et al*., 1999; Bekele, 2002).

The aim of this study was to investigate the seasonal egg output trends in a Mediterranean area, the Monti Livornesi Park (Tuscany region, Central Italy), in two wild ungulates, the mouflon (*Ovis ammon*) and the wild boar (*Sus scofa*).

STUDY AREA AND METHODS

The study area is located in the Monti Livornesi Park (43° 32' N, 10° 25') and covers approximately 50 km^2. The territory is hilly (prevalently 400 m a.s.l.) and crossed by several streams. The landscape is dominated by Mediterranean scrub with pine woods on the western side close to the coasts of the Tyrrhenian Sea and coppice forests towards to east. The climate is typically Mediterranean. During the study period mean temperatures ranged between 7.3°C in December and 27.4°C in July, and mean annual rainfall was 68 mm (Fig. 1). At the time of the study there were around 400 wild boars and 30 mouflons. The park is not fenced so that the animals can leave the protected area.

From February 1998 to January 1999, 21 samples of 30 g of faeces were collected monthly in the Park from both wild boars and mouflons. The number of samples is such that the probability of finding at least one positive sample is 95% when the unknown prevalence is supposed to be 13%. Each sample was labelled with the date of collection and preserved inside a disposable plastic bag for laboratory analy-

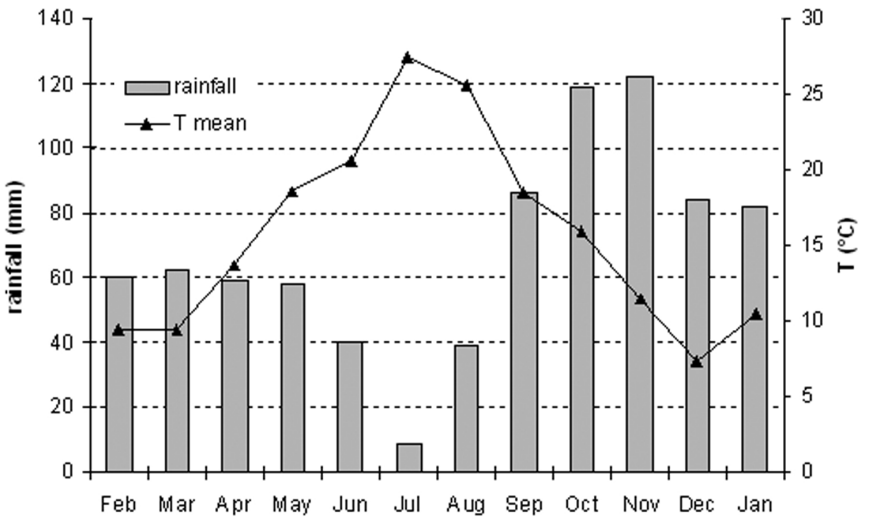

Figure 1 - Monthly rainfall and mean temperature during the study period. Meteorological data were obtained from the Weather Station of Livorno, situated approximately 10 km west from the centre of the park.

sis. Following MAFF (1986), the samples underwent a qualitative test by flotation in a NaCl saturated solution (density 1.200) and parasite eggs or oocysts were counted with a McMaster chamber, using a solution of $NaNO_3$ and $Na_2S_2O_3$ with density 1.440 (quantitative test). The number of eggs/g (EPG), for Coccidia OPG (oocysts/g), was determined. A threshold value of 20 eggs (or oocysts)/g was chosen as best balance between slide transparency and test sensitivity (MAFF, 1986).

Statistical analysis included following tests (Armitage et al. 2002; Yandell, 1997): a) χ^2 test, to verify the uniformity in time of monthly eggs output, b) Shapiro-Wilk test, to verify the normality of the eggs output distributions, c) Kruskal-Wallis test, to compare the monthly median EPG or OPG, d) Tukey HSD test with Duncan diagrams, to find the significant peaks of eggs output, e) nonparametric bootstrap resampling technique (Davison and Hinkley, 1997), to plan the temporal length of a future study, f) logistic regression (Hosmer and Lemeshow, 2000), to verify the correlation among egg output and climatic variations. The analysis was carried out using R 1.8.1 (R Development Core Team, 2003).

RESULTS

In the faeces of wild boars, eggs or oocysts of the following parasites were found: Coccidia (*Eimeria* spp.), gastro-intestinal strongyles, metastrongyles, *Trichuris suis*, Spirurides, *Ascaris suum*, *Dicrocoelium* spp. (Tab. 1). In mouflon faeces, oocysts of Coccidia (*Eimeria* spp), *Nematodirus* spp., other gastro-intestinal strongyles, strongyloides, *Dicrocoelium* spp., *Trichuris ovis*, *Moniezia expansa* and *M. benedeni* were detected (Tab. 2).

From the qualitative test, it turned out

that, in wild boars, oocysts of Coccidia, eggs of metastrongyles and of gastro-intestinal strongyles had the highest frequency (Tab. 1) as also recorded in mouflons for oocysts of Coccidia and eggs of gastro-intestinal strongyles (Tab. 2).

The distribution of monthly frequencies of wild boars and mouflons positive for parasite eggs were found to be all non uniform. The only exception was the distribution of mouflons with

Table 1 - Parasites of wild boars. In the "pos" columns are the numbers of samples positive to the qualitative test (first line) and above threshold in the quantitative test (second line). In the empty cells both values are 0. In the "OPG/EPG" columns are the median (first line) and the interquartile interval (second line) of the oocyst/egg output in the 21 monthly samples, according to the quantitative test. In the empty cells the median of OPG/EPG is 0, with interquartile interval 0-0. The number of samples positive at the quantitative analysis is reported, in order to account for possible distortions in the counting of light eggs (oocysts) because of the heavy solution used.

	Eimeria spp.		Intestinal strongyles		Meta-strongyles		*Trichuris suis*		*Spirurides*		*Ascaris suum*		*Dicrocoelium* spp.	
Month	pos	OPG	pos	EPG	pos	EPG	pos	EPG	pos	EPG	pos	EPG	pos	EPG
F 1998	15	60	3		2		1							
	15	0-80	0		2		1							
M	2		3		1									
	2		0		0									
A	18	120	13	20	12	20	3				2		2	
	17	40-240	12	0-80	11	0-80	1				1		1	
M	6		1				2		2					
	5		1				1		2					
J	9	0			10	0			7	0	10	0		
	8	0-100			9	0-40			7	0-20	10	0-440		
J	21	1660							5					
	21	1340-2640							4					
A	4				1									
	4				1									
S	18	100	1											
	18	60-120	0											
O	7	0	5				3		2					
	7	0-20	5				3		2					
N	3		1		1		4		1					
	2		1		1		4		1					
D	17	160	8	0	16	80	3							
	17	80-720	8	0-60	16	20-100	3							
J1999	10	0	2		11	20	5							
	10	0-40	1		11	0-120	5							

Table 2 - Parasites of mouflons. Abbreviations and explanations as in Table 1.

	Eimeria spp.		Intestinal strongyles		*Dicrocoelium* spp.		*Nematodirus* spp.		*Trichuris ovis*		*Moniezia* spp.		Strongy-loides	
Month	pos	OPG	pos	EPG	pos	EPG	pos	EPG	pos	EPG	pos	EPG	pos	EPG
F 1998	10	0	10				4		8				1	
	7	20-20	5				1		5				0	
M	6		5				3		4					
	5		1				2		2					
A	12	0	15	0			4							
	7	0-40	9	0-20			3							
M	11	0	6				1						2	
	8	0-20	3				0						1	
J	13	20	9										1	
	12	0-60	3										1	
J	21	160	14	0	2						8	0		
	19	60-360	10	0-20	2						8	0-200		
A	20	120	9											
	20	80-160	5											
S	8	0	9	0										
	8	0-60	7	0-20										
O	16	60	10	0										
	16	20-100	7	0-20										
N	20	40	5											
	20	40-100	5											
D	16	100	2										1	
	16	20-140	1										1	
J1999	15	20	17	140	15	40							2	
	11	0-20	15	0-220	14	0-60							1	

oocysts of Coccidia which did not differ significantly from a uniform distribution (P = 0.070). In this case the annual prevalence turned out to be 67% (95% confidence interval 60% - 72%). The quantitative test showed that for all parasites the monthly egg output was highly non uniform, as was observed in the case of the five most frequent parasite species mentioned above (2 for mouflons and 3 for wild boars). The distribution of the data was found to be non normal (Shapiro-Wilk test, P < 0.001). Monthly median EPG were compared in all five cases and highly significant differences were found (Kruskal-Wallis test, P < 0.001). The origin of the differences was highlighted by dividing months into subgroups corresponding to different levels of eggs output (Tab. 3). For instance, the Duncan diagram of the gastro-intestinal strongyles in wild boars shows a level of high output in December–April and one of low output from February to October. The months of October and

Table 3 - Duncan diagrams where a line connects months belonging to the same significant level of egg output.

Wild boar

Coccidia

nov	mar	aug	may	oct	jan	jun	feb	sep	apr	dec	jul

Intestinal strongyles

feb	mar	jun	jul	aug	sep	jan	nov	may	oct	dec	apr

Metastrongyles

mar	may	jul	sep	oct	nov	aug	feb	jun	apr	jan	dec

Mouflon

Coccidia

mar	may	feb	apr	jan	sep	jun	oct	nov	dec	jul	aug

Intestinal strongyles

mar	dec	jun	may	aug	nov	feb	oct	sep	apr	jul	jan

December belong also to an intermediate level of output. In order to reach a 95% probability of separating levels completely, a five times larger amount of data would be needed, as found with a nonparametric bootstrap resampling technique.

Monthly prevalences were found not significantly dependent on meteorological factors (temperature and rainfall) in the cases of Coccidia, both in mouflons and in wild boars, and intestinal strongyles in mouflons (logistic regression, lowest P value 0.18). For gastro-intestinal strongyles and metastrongyles in wild boars the dependence of monthly prevalence on mean temperature is significant, but the result is invalidated by data dispersion (goodness-of-fit, $P < 0.001$).

DISCUSSION

Almost all the observed parasites are common in Italian wild boars and mouflons. *M. expansa* was already reported in mouflons in Italy (Garippa, 1994) while, according to the national literature, *M. benedeni* was not seen before.

For wild boars oocyst output was highest in July and December following a climatic period of high humidity (spring and fall) that favours the sporulation and the resistance of the oocysts in the environment. In July this mechanism should be enforced by the presence of the litters, more susceptible to infection and therefore good parasite producers. Egg output of gastro-intestinal strongyles was highest in April and December.

The peak in the first period suggests the presence of a periparturient rise, a particular phase of the host–parasite relationship that favours young piglet infection (Bronson, 1989; Jeffcoate *et al.*, 1992). On the other hand this spring rise could be interpreted as the renewed activity of the infective stages (L3) after a period of winter rest (Parnell *et al.*, 1954; Paver *et al.*, 1955).

For metastrongyles the favoured months were April, December and January in connection with high humidity which favours the activity of the intermediate host (earthworm). The considerations made for gastro-intestinal strongyles should be done also for the lungworms. December turned out to be an especially favoured month for egg/oocyst output of all parasites. This can be interpreted as a long tailed autumn rise connected to the mildness of the climate, and it represents the most unexpected result of the study, but may reflect an adaptive parasite answer to Mediterranean climate. Resistance stages are especially endangered by summer dryness and may be able to resist in winter. In June there are several samples positive for *Ascaris suum*, a parasite with a direct cycle. Resistant eggs after output can reach the infective stage on the soil in 4-6 weeks of sufficiently high temperature (about 20° C). This happens in the study area from May on. Ascarids are present especially in young animals which can produce a large amount of eggs and may activate a cycle of spring infection patently observed in summer.

In the case of mouflons, which were about 30 animals, the collection of 21 samples monthly, the same number as for wild boars (400 animals), may be biased by the sampling procedure that should favour the presence of more samples of the same animal. Late spring is the lambing period for mouflon ewes, so that a concurrent periparturient rise of egg output was expected also in this species. In the present research there is no evidence of such a phenomenon. Another rise of egg output was expected for mouflons in summer (July–August) due to the contribution of newly infected lambs. This trend is confirmed both for Coccidia and gastro-intestinal strongyles.

Some remarks can be made for parasites with an indirect life-cycle. For *Moniezia* spp. the intermediate hosts (mites) are active from March. The parasites develop inside them for 6-8 weeks to the infective larval stage. Another 4-6 weeks are needed before adult worms are ready to lay eggs. Thus the high prevalence in July is well timed.

For *Dicrocoelium* spp., parasites with two intermediate hosts (first slugs and then ants), one observes a January peak. The final host is infected when it ingests ants with metacercariae. Such ants, when the temperature is low, remain attached to grass stems. In the study area this can happen during September nights. The parasites need two and a half months inside the final host to be ready for egg laying. December and January are therefore months in which a rise of egg output was observed and justified.

In the Mediterranean region, such as the one considered, the peaks of egg-oocyst output appear in different months according to the different para-

site biology and survival strategies linked to climatic phenomena.

In our study area, wild ungulates have a low parasite egg-oocyst dropping that, considering the low prevalence values, may suggest a moderate parasite burden. This is probably due to overdispersion that does not allow density dependent phenomena. Coccidia (*Eimeria* spp.) is the most represented group, but clinical outbreaks in wild mammals are very rare.

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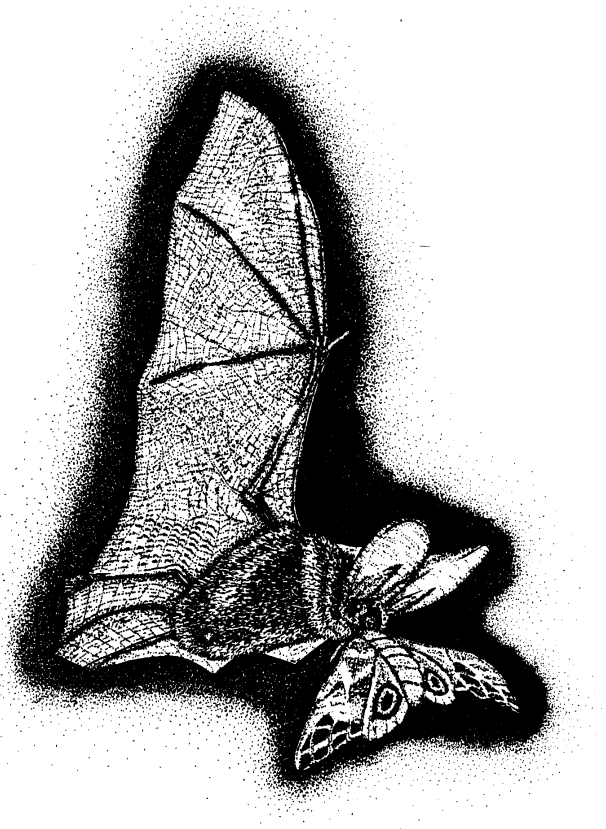

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