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Coccidia in Hare (*Lepus europaeus*) Reared in Umbria, Italy: Bioepidemiological Study

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ABSTRACT

A study to elucidate the main species of coccidia was carried out on breeder hare reared in an Umbrian intensive game breeding farm and on juvenile hare reared first in cages and subsequently in 9 protected areas in the Province of Perugia (Italy). A total of 2,100 faecal samples from game hare were collected monthly between January 1990-December 1993. Results showed that coccidia were not present in the breeder and juvenile hare kept in cages; conversely, *Eimeria leporis* and *E. semisculpta* were detected in the juvenile hare reared in eight of the protected areas but showed a wide range of variability. In addition *E. robertsoni*, *E. townsendi*, *E. hungarica* and *E. europea* were isolated from the dead hare reared in one protected area with a high coccidial prevalence.

INTRODUCTION

Coccidiosis is considered to be a most frequent parasitic infection both in wild and in farmed hare. The earliest reports on the etiology of coccidiosis in the genus *Lepus* date back to 1923 (Nieshultz). The Eimerian parasites occurring in the species of the genera *Lepus*, *Sylvilagus*, and *Oryctolagus* were first morphologically differentiated by Carvalho (1943) and subsequently their host-specificity was reported by Pellerdy (1956). These studies based on cross-infection experiments clarified much of the earlier confusion by providing a detailed assessment of previous reports on leporine coccidia and describing seven host-specific species of *Eimeria* from the hare (*Lepus europaeus*) (Davies 1963).

Epidemiological and etiological studies on coccidiosis both in wild and farmed hare have been carried out in several countries (Pastuszko 1961; Novak et al. 1966; Bouvier 1967; Golemansky 1975; Kutzer and Frey 1976; Shellner 1979; Soveri and Valtonen 1983; Chorust 1984; Allgower 1989). Italian reports have described the status of this parasitosis in hare reared in various regions (Ballarini 1966; Francalanci and Manfredini 1970; Gallazzi et al. 1983, 1990; Leoni et al. 1986; Poli et al. 1988; Ravajoli et al. 1988; Terracciano et al. 1988; Camarda et al. 1989; Mazzoni della Stella et al. 1991; Sacchi and Prigioni 1991; Verdone et al. 1991; Zanni et al. 1992; Poglayen et al. 1994). However, little is known about the incidence of coccidiosis in hare reared in Umbria except the short reports by Tacconi et al. (1992, 1993), and no reports on the species of *Eimeria*, harboured in these hare, have been published to date.

The aim of the present study is to characterize taxonomically the species of *Eimeria* isolated from the hare reared in Umbria, and to define better the prevalence of coccidiosis in these hare.

MATERIALS AND METHODS

This study began in 1990 in order to detect the prevalence of the genus *Eimeria* in the breeders reared in cages in a public game farm (Torre Certalta - Umbria - Italy) and in the juvenile hare reared from 30 to 75 days of age in cages (Torre Certalta) and then put in protected areas until 110 days of age. Afterwards the juvenile hare were released for restocking of wild-life reserves.

Breeders

Seven hundred and fifty faecal samples from 50 braces, reared in separate wooden cages (Torre Certalta), were collected between January 1990 to December 1993. The collection of samples was carried out monthly.

Juvenile hare

A total of 1,350 faecal samples were collected between January 1990-December 1993. Of them 117 were collected from the hare when reared in separate wooden cages (Torre Certalta), and 1,233 were collected from the hare when living in protected areas. The collection of the samples was carried out monthly.

Environment

Nine protected areas (called C-K), each consisting of about 1 ha (60% cultivated beans, cereals, sunflowers; 40% dense bush and woodland) were set up in Umbria (Table 1). Pellets with coccidiostats and water were supplied ad libitum in each area. To avoid the admission of domestic and wild animals, the areas were surrounded by two layers of nets 150 cm in height with 12 mm holes and with 50 cm of the net underground.

Table 1 Environmental characteristics of the nine protected areas

Protected Areas	
C	Flat, sunny area with grassland
D	Very hilly, well-drained rocky area
E	Hilly area with pastures and bushland
F	Slightly sloping, wet, hilly area with grassland and without stones
G	Flat, wet area
H	Mostly flat, well-drained area - 1/4 woodland
I	Very hilly area with various crops, grass land and 1/4 woodland (the largest area)
J	Very hilly area with pastures and bushland
K	Flat, wet area near lake with pastures and morning dew

Parasitological examination

Faecal samples (each sample consists of between three to four pellets, taken from different points within each cage and within each area) were floatated with NaCl-hypersaline solution (s.w. 1,200) and the species of coccidia were identified based on the oocyst morphology and minimum time for sporulation as described by Carvalho (1943) and Pellerdy (1965).

Post-mortem examination

Four dead hare from area F were submitted to our laboratory for pathological and parasitological findings during the summer of 1993.

RESULTS

Prevalence for coccidia in all faecal samples is indicated in Tables 2 and 3. The morphological characteristics and sporulation times of oocysts are shown in Table 4. The results can be summarized as follows:

1) All the faecal samples collected from the braces and caged juvenile hare were negative for coccidia (Table 2).

2) Coccidia were found in the faecal samples collected from juvenile hare in the protected areas; only one protected area (C) was coccidia-free while the remaining showed various degrees of coccidial prevalence (Table 3).

Table 2. Positivity for coccidia in faecal samples from breeders and juvenile hare kept in cages (January 1990-December 1993)

Hare	No of animals examined	No of faecal samples examined	Total positivity %
Breeders	100	750	0
Juvenile hare	350	117	0
Total	450	867	0

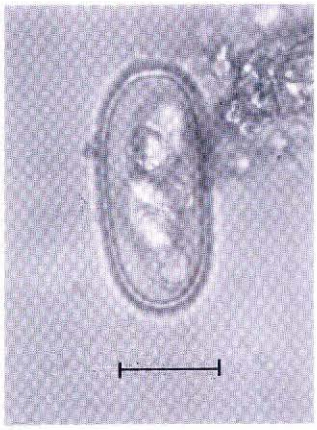

Fig.1. Sporulated oocyst of Eimeria leporis. Bar 10 μm.

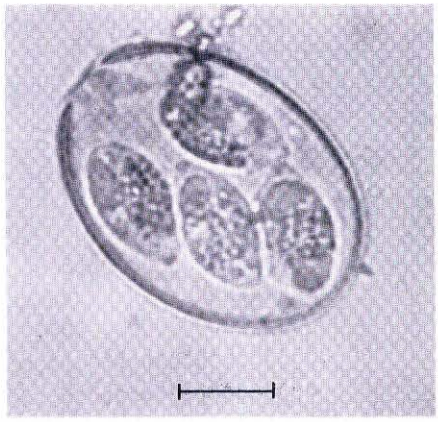

Fig.2. Sporulated oocyst of Eimeria semisculpta. Bar 10 μm.

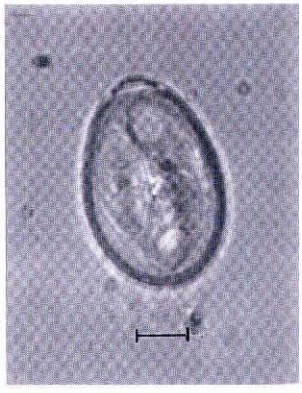

Fig.3. Sporulated oocyst of Eimeria robertsoni. Bar 10 μm.

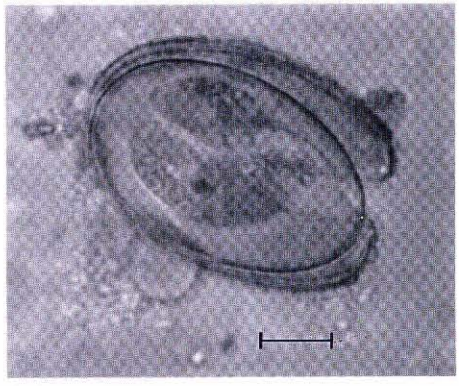

Fig.4. Sporulated oocyst of Eimeria townsendi. Bar 10 μm.

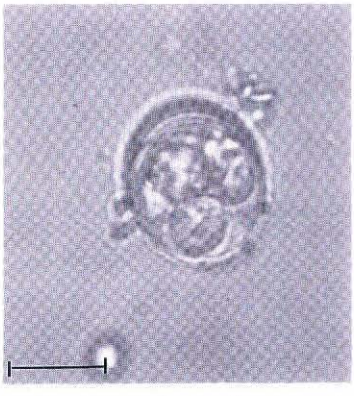

Fig.5. Sporulated oocyst of Eimeria hungarica. Bar 10 μm.

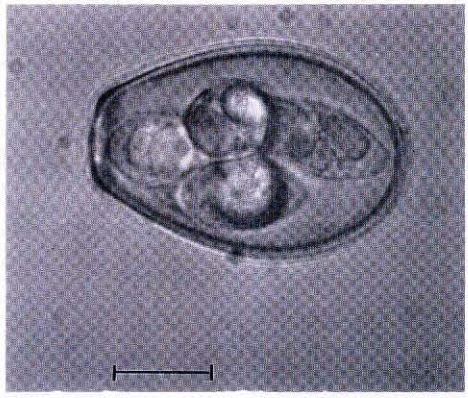

Fig.6. Sporulated oocyst of Eimeria europea. Bar 10 μm.

Table 3. Positivity for coccidia in juvenile hare faecal samples* in protected areas (January 1990-December 1993)

Protected areas	Positivity degree (%)**				Total positivity
	+	++	+++	++++	(%)
C	0.0	0.0	0.0	0.0	0.0
D	16.6				16.6
E			10.0		10.0
F		12.6	18.0	12.2	42.8
G		14.8		35.2	50.0
H	30.0	13.0	14.1		57.1
I	100.0				100.0
J	50.0		50.0		100.0
K		35.0	65.0		100.0

* No of total faecal samples examined = 1,233 (No of total hare examined = 1,263)
** + : oocyst count/gr (OPG) = ≤ 50,000
++ : oocyst count/gr (OPG) = 50,000-150,000
+++ : oocyst count/gr (OPG) = 150,000-300,000
++++ : oocyst count/gr (OPG) = ≥300,000

Table 4 Morphological characteristics and sporulation time of oocysts found from the faecal samples examined

Parameters	*E. leporis*	*E. semisculpta*	*E. hungarica*	*E. townsendi*	*E. robertsoni*	*E. europea*
Shape	elongated	ellipsoidal	round	ovoid	ovold	ellipsoidal
Wall	thin	thick	thin	smooth	thick	thin
Wall colour	colourless	yellowish brown	colourless	yellowish	yellowish	colourless
Micropyle	absent	present	absent	absent	present	present
Size (µm)	28-38 by 16-20	35-45 by 22-27	13-15 by 12-14	36-44 by 25-31	34-52 by 23-32	26-34 by 15-20
Sporulation time	2-3 days	3-4 days	2 days	8 days	3-4 days	3-4 days
Residual body of oocyst	present	absent	present	absent	present	present

3) Two species of *Eimeria* were isolated from all the positive faecal samples. The species were identified as *E. leporis* (Fig. 1) and *E. semisculpta* (Fig. 2). Their characterization is shown in Table 4.

4) Four further species of *E.* were detected from the intestinal contents of the four dead hare in area F. These were identified as *E. robertsoni, E. townsendi, E.*

hungarica and *E. europea* (Figs. 3, 4, 5, and 6). Their characterization is reported in Table 4. A quantitative evaluation of each single species proves to be difficult, since some were clearly identifiable only after sporulation.

5) The post-mortem examination of the four dead hare showed severe enteritis as the cause of death. No lesions caused by coccidiosis were found in the livers of these game.

6) In all the hares examined the highest prevalence of coccidia was observed in the late summer-early autumn period.

DISCUSSION

Good health status represents one of the crucial factors for the success in restocking European hare (*Lepus europaeus*). It is essential to improve their capability to overcome negative factors such as adverse environmental or weather conditions, and physical stress causing varying degrees of impairment of normal body functions. Parasites play an important role in the pathology of hare in that as bioregulators they can influence the dynamics of the population. Special emphasis should be given to the clinical form and in particular to the subclinical form of coccidiosis which occurs frequently in both wild and free-living hare. The results of our investigation highlighted two main aspects:

1. Breeders and juvenile hare, kept in cages, were negative for coccidia: this is probably due to both the good management conditions and the use of pellets with coccidiostats as food. The passage in the protected areas and the subsequent contamination with related coprological positivity, can arise from the different feeding and/or the environmental conditions of these areas and/or the possible contamination by free living hare allocated in neighbouring wild life reserves. This contamination may have occurred before the utilization of this territory for protected areas. On the contrary, the responsability of wild rabbits in the epidemiology of this parasitic disease seems irrelevant owing to the host-specificity of the genus *Eimeria*, and the protection given by the nets against these animals. A wide range of variability for coccidia positivity was recorded in the eight areas (the protected area C was coccidia-free) and the highest levels of total OPG count were observed from the end of summer to the beginning of autumn when weather conditions (temperature and humidity) are most favourable for oocystic sporulation. This season-related prevalence is in agreement with the studies conducted by Terracciano et al. (1988) and Mazzoni della Stella et al. (1991).

2. *E. leporis* and *E. semisculpta* were isolated from all positive faecal samples. *Eimeria leporis* have been recorded frequently in Italy (Francalanci and Manfredini 1970; Terracciano et al. 1988; Mazzoni della Stella et al. 1991; Sacchi and Prigioni 1991) while *E. semisculpta* was observed in Tuscany (Terracciano et al. 1988;

Mazzoni della Stella, 1991) and in Veneto (Francalanci and Manfredini 1970) to date. It is noteworthy that four further species of *Eimeria* such as *E. robertsoni, E. townsendi, E. hungarica* and *E. europea* were identified from the intestinal contents of the four dead hares. The absence of these species in the faecal samples of living hares is considered to be due to the low number of parasites in their intestinal contents and to intermittent oocystic emission. Since the main purpose of the present study was to morphologically characterize *Eimeriae* in Umbrian hare reared in captivity, more detailed investigations are required for a better definition of the numerous behavioural aspects of *Eimeria-Lepus* cenosis especially with regards to the coexistence of other parasites that might influence both the pathology and the immunology status of these hare. In conclusion, rigid prophylactic measures must be taken in the management of the areas where the hare are put in order to curb the diffusion of parasites, thus improving the health status of hare for repopulation.

REFERENCES

Allgower, R. 1989. Einige okologische Aspekte des Parastenbefalles beim Feldhasen. pp.224-229. *In:* Transactions of the XIX Congress of the International Union of Game Biologists,Trondheim, Norwey.

Ballarini, G. 1966. Quantitative aspects in the dynamics of Eimeridae populations in cenosis with *Lepus europaeus* (space of the intestinal tract). *Veterinaria Italiana, 17:* 252-262.

Bouvier, G. 1967. Les Coccides rencontrees en Suisse chez le lievre gris (*Lepus europaeus*). *Ann. Parasitol. Hum. Comp.,42:* 551.

Camarda, A., Ianieri, A. & Oreste, E. 1989. Patologia spontanea in avifauna selvatica e leporidi allevati con metodo intensivo. pp.115-126. *In:* Atti XI Convegno Umbriacarni 1989. Bastia Umbra 9-10 Giugno.

Carvalho, J. C. M. 1943. The coccidia of wild rabbits of Jowa. I. Taxonomy and host-specificity. *Jowa Sta. Coll. J. Sci. 18:* 103-134.

Chorust, K. 1984. Dynamic of coccidial infection in free living and cage-reared European hares. *Acta Vet. Brno 53:* 152-175.

Davies, S. F. M., Joyner, L. P. & Kendall, S. B. Coccidiosis. pp.152-154. *In:* Oliver and Boyd (Ed) Edinburg and London 1963.

Francalanci, G. & Manfredini, L. 1970. Ricerca degli agenti specifici della coccidiosi della lepre (*Lepus europaeus*). *La Clinica Veterinaria 93:* 130-135.

Gallazzi, D., Genchi, C., Manfredi, M. T., Rampin, T., & Traldi, T. 1983. Infezioni parassitarie nelle specie minori. *IZ 5:* 104-108.

Gallazzi, D., Rota, T. & Facchin, E. 1990. Cause di malattia e morte nelle lepri del Nord Italia. pp.221-235. Gestione e Protezione del Patrimonio Faunistico.

Atti I e II Corso di Aggiornamento. Brescia 1989-1990.

Golemansky, V. 1975. On the Coccidia (Sporozoa, Eimeriidae) of the European Hare (*Lepus europaeus* L.) in Bulgaria. *Acta Zoologica Bulqarica, 3:* 39-47.

Kutzer, E. (Von), & Frey, H. 1976. Die parasiten der felhasen (*Lepus europaeus*) in Osterreich. *Berl. Munch. Tierarztl. Wschr. 89:* 480.

Leoni, A., Grippa, G., & Arru, E. 1986. Principali endoparassiti dei leporidi selvatici (Orynctolagus cuniculus e Lepus mediterra neus) in Sardegna. *Ann. Ist. Super. Sanita 22:* 501-504.

Mazzoni della Stella, R., Guberti, V., Farnetani, M. & Trocchi, V. 1991. Stagionalita e influenza della densita di popolazione nella emissione di oocisti e uova di parassiti intestinali della lepre (Lepus europaeus). pp.785-786. In: Pubblicazioni Istituto di Biologia della selvaggina "Alessandro Chigi". Posters N°17.

Nieschulz, O. 1923. Uber Hasenkozidien (*Eimeria leporis* n. sp.). *Deutsch. Tieraztl. Wochscr.31:* 245-247.

Novak, J., Dyk, V. & Zavadil, R. 1966. Die Herst parasitenfauna der Hasenverdaungsorgane in Sudmahren und der Sudslowakei. *Sb. vys. Sk. Zemed. Ies. Fac. Brno,14:* 48.

Pastuszko J. 1961. The occurrence of Eimeriinae Wenyon in the hare in Poland. *Acta Parasitologica Polonica 9:* 23.

Pellerdy, L. P. 1956. On the status of the *Eimeria* species of *Lepus europaeus* and related species. *Acta Vet. Acad. Sc. Hungsr.* 6, Budapest.

Pellerdy, L. P. Coccidia and Coccidiosis. pp.1-657. Akademiai Kiado. House of the Hungarian Academy of Sciences (Ed) Budapest 1965.

Poglayen, G., Roda, R., Zanni, M.L., Amendola, B. & Pepa, M. 1994. Parassiti dell'apparato digerente della lepre (*Lepus europaeus*) nelle province di Bologna e Bolzano. *Selezione Veterinaria 35:* 193-199.

Poli, A., Mancianti, F., Trocchi, V., Verdone, M. & Terracciano, G. 1988. Influence of population density on seasonal variations of parasitic infection in European hare (*Lepus europaeus* Pallas). pp.313-319. Verhanlungsbericht des Internationalen Symposiums uber die Erkrankungen der Zoo-und Wildtiere. Sofia.

Ravajoli, C., Giovannini, A., Trocchi, V. & Giunchi, G. 1988. Indagi ne sulla gestione della Lepre europea (*Lepus europaeus* Pallas, 1778) nelle zone di ripopolamento e cattura della provincia di Forli. Amministrazione Provincia Forli.

Sacchi, L. & Prigioni, C. 1991. Prevalenza e carica parassitaria da coccidi e nematodi in lepri campionate in aree protette della provincia di Pavia. pp.787-788. In: Atti II Convegno Nazionale Biologia della Selvaggina, Bologna 1991.

Supplemento.

Shellner, H. P. 1979. Causes of death among wild hares in Bavaria. *Berliner und Munchener Tierarztliche Wochenschrift, 92:* 302-303.

Soveri, T. & Veltonen, M. 1983. Endoparasites of hares (*Lepus timidus* L. and *Lepus europaeus* Pallas) in Finland. *J. Wildl. Dis. 19:* 341-387.

Tacconi, G., Moretti, A., Piergili-Fioretti, D. & Nobilini, N. 1992. Parasitic conditions in game breeding farms in Central Italy. p.393. *In:* Proceedings of the XIX World's Poultry Congress Amsterdam, 20-24. September 1992.

Tacconi, G., Piergili-Fioretti, D., Moretti, A., Nobilini. N. & Pasquali, P. 1993. Incidence of coccidia in hare (Lepus euro paeus) in Central Italy. p.125. *In:* Abstracts of the IX International Congress of Protozoology. July 25-31. Berlin.

Terracciano, G., Mancianti, F. & Marconcini, A. 1988. Indagine paras sitologica nella lepre. *Summa 4:* 217-220.

Verdone, M., Terracciano, G., Trocchi, V. & Poli, A. 1991. Dinamica di popolazioni di Lepre (*Lepus europaeus*, Pallas), prevalenza ed intensita delle parassitosi. pp.637-640. *In:* Atti II Seminario Italiano censimenti faunistici dei verte brati. Supplemento alle Ricerche di Biologia della Selvaggina, XVI.

Zanni, M. L., Capucci, L., Fabbi, M., Giovannini, S., Lavazza, A., Tagliabue S., Poglayen, G., Roda, R. & Tasselli, A.1992. Programma di monitoraggio sanitario nella lepre (*Lepus europaeus*) in provincia di Ravenna. *Selezione Veterinaria 10:* 1103-1108.