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EXPERIMENTAL INFECTION OF EIGHT SPECIES OF HELICIDAE (MOLLUSCA) USING *CYSTOCAULUS OCREATUS*

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SUMMARY

The susceptibility of eight species of Helicidae molluscs to *C. ocreatus* was studied under experimental conditions.

The statistical study was based on analysis of variance of the % of penetration, evolution and L III, and subsequently the corresponding correlation coefficient was obtained between them. Student's "t" was used to find out the existence or not of significative dfferences between the averages of the above mentioned parametres for each species.

We took into account fundamentally the evolution and L III percentages, the days when the first L III appeared and when all had reached this final stage. The following list shows, in decreasing order, the most adequate species as intermediate hosts: *C. (C.) virgata, M. (A.) granulata, H. ordunensis, C. (X.) cespitum arigonis,* y *C. barbara.* In our opinion *H. itala, O. (O.) barbula* and *C. nemoralis* are not favourable I.H.

Key Words: Protostrongylinae, *C. ocreatus*, experimental infection, intermediate hosts, land snails, Helicidae.

RESUMEN

Se estudia la receptividad experimental a *C. ocreatus* de ocho especies de moluscos de la familia Helicidae.

El estudio estadístico se basó en el análisis de varianza del % de penetración, evolución y L III, entre los que se estableció, en cada caso, el correspondiente coeficiente de correlación. Se empleó la "t" de Student para la constatación de la existencia o no de diferencias significativas, entre las medias de los parámetros anteriormente mencionados, para cada especie de molusco.

Considerando fundamentalmente el % de evolución y el de L III, así como el día de aparición de las primeras L III y, aquel en el que todas se encontraban en este estado, se pueden considerar como especies más adecuadas, en orden decreciente: *C. (C.) virgata, M. (A.) granulata, H. ordunensis, C. (X.) cespitum arigonis,* y *C. barbara.* A nuestro juicio, no se pueden señalar como H.I. adecuados a *H. itala, O. (O.) barbula* y *C. nemoralis.*

Palabras Clave: Protostrongylinae, *C. ocreatus,* infestación experimental, hospedadores intermediarios, moluscos terrestres, Helicidae.

INTRODUCTION

Cystocaulus ocreatus (RAILLIET & HENRY, 1907) has been frequently mentioned as an ovine parasite in Spain (CORDERO et al., 1980).

Helicidae are among its intermediate hosts (I.H.). Several aspects concerning its natural and/or experimental infection have been studied in this country by TARAZONA (1955), RAMIREZ (1967) and MANGA et al. (1979) and MANGA & MORRONDO (in print).

This paper studies the susceptibility of eight species of Helicidae molluscs mentioned in León by MANGA (1977), to *C. ocreatus.* New data have been obtained for some of these species, adding to those already observed by RAMIREZ (ibid.) and CABARET & DAKKAK (1979).

MATERIALS AND METHODS

The larvae I (L-I) were obtained from faeces of a sheep which had undergone pure, natural infection by *C. ocreatus.*

The species of Helicidae used were: *Cernuella (Cernuella) virgata* (DA COSTA, 1778), *Cernuella (Xeromagna) cespitum arigonis* (SCHMIDT, 1853) *Helicella itala* (L., 1758), *Helicella ordunensis* (KOBELL, 1882), *Cochlicella barbara* (L., 1758), *Monacha (Ashfordia) granulata* (ALDER, 1830), *Oestophora (Oestophora)*

barbula (ROSSMASSLER, 1838), *Cepaea nemoralis* (L., 1758), collected in one of the places mentioned by MANGA (1977). Ten per cent of the specimens were examined to check for the absence of natural infection. A total of 362 adult molluscs were infected (see table 1).

The infection and handling of the snails were carried out according to MORRONDO & MANGA (in print.) and MORRONDO et al. (in print.).

In order to find out if there were significant interspecific differences among the species tested with *C. ocreatus* the corresponding analysis of variance (one way) with $p \leq 0.001$ were performed using a Hewlett-Packard-97 calculator.

The three parametres studied were: a) the percentage of larvae which penetrated, b) their degree of evolution and c) the percentage which reached L-III. After, Student's "t" (with the value of $p = 0.05$) was applied to find out the existence or not of significative differences between the averages of each species in these parametres.

The corresponding correlation coefficient was also studied to ascertain if there was any relationship between the parametres.

RESULTS AND DISCUSSION

The results in table 1 for *H. itala, C. (X.) cespitum arigonis, C. (C.) virgata, C. barbara* y *C. nemoralis* show that our larvae/mollusc average during the experimental period was lower than that obtained by RAMIREZ (1967) for the same species. DAVTIAN (1949) also obtained a lower larvae/mollusc minimum and maximum in other Helicidae.

Compared to RAMIREZ's results our *C. ocreatus* reached the L III stage in *C. nemoralis* and *C. barbara* in less time, although this author infected with a plurigenic mixture of L-I from the lung.

However, our results coincide with those of CABARET & DAKKAK (1979) about the time required for *C. barbara* and a mixture of parasites like the above mentioned, but from faeces.

TABLE 1

Molluscs	N.° of infect. specimens	Av. temp. (°C)	N.° LI per mollusc	N.° larvae per molluscs			Day LII appeared	Day LIII appeared	Day LIII all
				min.	max.	$\bar{x}$			
C. (C.) virgata	43	18	250	1	94	8.4	10	17	25
M. (A.) ganulata	27	20	320	2	105	33.5	9	32	49(70% LIII)
H. ordunensis	37	18	210	1	16	3.8	10	24	29
C. (X.) cespitum arigonis	50	20	150	1	30	4.2	10	17	25
C. barbara	52	18	310	1	42	15.8	10	28	35
H. itala	40	18	200	1	50	4.2	9	30	42
O. (O.) barbula	30	18	200	1	9	2.3	9	37	42(91% LIII)
C. nemoralis	83	18	525	1	24	3.3	10	28	44

TABLE 2

Measurements LII of *C. ocreatus*	Own measurements (19 L.)			Joyeux & Gaud (1946)			Gerichter (1951)	Tarazona (1955) (10 L.)		
	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$		min.	max.	$\bar{x}$
Total length	585.0	676.5	631.6	350	583	490.3	630	455	632	582.0
Length of oesophagus	171.4	622.8	211.4	145	195	164.7	206	160	204	176.8
Anterior end to nerve ring	94,3	114.3	104.3							
Anterior end to excretory pore	102.8	131.4	114.0	72	115	89.4				
Posterior end to anus	51.4	57.1	54.2	30	45	37.6	50-55	29	54	43.0
Posterior end to genital primordium	214.3	277.1	240.0	150	275	196.8				
Total length/length of oesophagus	2.2	3.6	3.0							
Total length/anterior end to nerve ring	5.1	6.6	5.9							
Total length/anterior end to excretory pore	4.5	6.0	5.5							
Total length/posterior end to anus	10.8	12.8	11.7							
Total length/posterior end to genital primordium	2.1	2.6	2.6							

TABLE 3

Measurements LIII of *C. ocreatus*	Own measurements (37 L.)			Joyeux & Gaud (1946)			Gerichter (1951)	Tarazona (1955) (10 L.)			El-Moukdak (1978)
	min.	max.	$\bar{x}$	min.	max.	$\bar{x}$		min.	max.	$\bar{x}$	
Length with cuticular sheat	747.0	1029.4	890.0								
Total length	655.9	957.1	748.3	600	740	660.0	750-790	536	726	626.2	592-598
Length of oesophagus	171.4	302.8	223.1	150	200	172.5				211.6	180-260
Anterior end to nerve ring	91.4	125.7	102.2								
Anterior end to excretory pore	111.1	174.3	137.1	80	160	106.8		102	116	109.7	110-168
Posterior end to anus	51.4	65.7	55.1	28	56	42.9	54-62	32	79	60.4	
Posterior end to genital primordium	228.6	348.6	280.9	185	285	227.5					
Total length/length of oesophagus	2.6	4.3	3.4								
Total length/anterior end to nerve ring	6.0	7.8	6.9								
Total length/anterior end to excretory pore	5.0	7.0	5.7								
Total length/posterior end to anus	11.5	17.8	13.5								
Total length/posterior end to genital primordium	2.0	3.1	2.7								

There is also some similarity, concerning time, to the data obtained by DAVTIAN (1940, 49), AZIMOV et al. (1975) using different *Helicella* species.

Our species of *Monacha* and *Helicella* took longer to reach L-III than those used by GERICHTER (1951), although he kept his specimens at a higher temperature (20-30°C).

We coincide with TARAZONA (1955) in the time the first L-II and L-III took to appear in *C. (C.) virgata* (called *Helicella (Cernuella) variabilis* var. *suberis* by him.).

It is normal to find different larval stages in the same snail, as proved in earlier work, MORRONDO et al. (in print) and MORRONDO & MANGA (in print)

As the L-II and L-III measurements (tables 2, 3) of all species were similar they were grouped together. In general, our data are similar to those of GERICHTER (1951), TARAZONA (1955) and EL-MOUKDAK (1978), but not to those of JOYEUX & GAUD (1946).

The three analysis of variance carried out (table 4) show highly significative interspecific differences among the eight species.

Bearing in mind the average percentage of the L-I penetration of *C. ocreatus* (table 5), the most suitable species appear in decreasing order: *C. nemoralis, O. (O.) barbula,* and *M. (A.) granulata, H. itala, C. (X.) cespitum arigonis, C. barbara, H. ordunensis,* and *C. (C.) virgata.* However, using Student's "t" it is impossible to find significant differences between *C. (C.) virgata* and *H. ordunensis, O. (O.) barbula* and *M. (A.) granulata, H. itala* and *C. nemoralis.*

As for the average percentage of evolution (table 5) the most suitable species were, in decreasing order: *C. (C.) virgata, M. (A.) granulata, C. barbara, H. ordunensis, C. (X.) cespitum arigonis, H. itala, O. (O.) barbula,* and *C. nemoralis.* On applying Student's "t" no significant differences were found between: *C. (X.) cespitum arigonis* and *C. barbara*; *H. itala* and *H. ordunensis*; *C. (C.) virgata* and *M. (A.) granulata*; *H. itala* and both *H. ordunensis* and *C. nemoralis*; *O. (O.) barbula* and both *H. itala* and *C. nemoralis.*

TABLE 4

ANALYSIS OF VARIANCE

Source of variation		S.S.	df	M.S.	F.
Species	% P.	131502.59	7	18786.08	91.80 +++
	% E.	10522.17	7	1503.11	11.82 +++
	% LIII	5217.76	7	745.39	6.24 +++
Error	% P.	72441.05	354	204.64	
	% E.	45130.74	355	127.13	
	% LIII	15895.00	133	119.51	
Total	% P.	203943.64	361		
	% E.	55652.91	362		
	% LIII	21112.76	140		

P. = penetration E. = evolution +++ = $p \leq 0.001$

TABLE 5

Molluscs	% Penetration		% Evolution		% LIII	
	$\bar{x}$	N.° observations	$\bar{x}$	N.° observations	$\bar{x}$	N.° observations
C. (C.) virgata	26.07	43	16.18	43	17.74	19
M. (A.) granulata	76.41	27	15.30	27	14.59	9
H. ordunensis	31.91	37	7.53	37	13.39	13
C. (X.) cespitum arigonis	61.26	50	5.63	50	9.43	16
C. barbara	54.92	52	9.18	52	8.66	26
H. itala	72.13	40	3.03	40	1.86	15
O. (O.) barbula	77.00	30	1.40	30	1.15	8
C. nemoralis	78.95	83	0.83	83	1.09	35

In the same way the most suitable species concerning the average percentage L-III (table 5) were: *C. (C.) virgata*; *M. (A.) granulata*; *H. ordunensis*; *C. (X.) cespitum arigonis*; *C. barbara*; *H. itala*; *O. (O.) barbula*, y *C. nemoralis*. However, it is impossible to affirm the existence of significant differences between: *C. (X.) cespitum arigonis* and *C. barbara*; *M. (A.) granulata* and both *H. ordunensis* and *C. (C.) virgata*; *O. (O.) barbula* and both *H. itala* and *C. nemoralis* using Student's "t".

Comparing the average percentages of penetration, evolution and L-III no correlation was observed between the first two, nor between the first and last, since the r^2 values were 0.29 and 0.53 respectively. Comparing the second and the last they appear to be correlated since r^2 was 0.85.

Bearing in mind that the percentages of evolution and of L-III are important in defining susceptibility of molluscs, we can affirm that highly significative interspecific differences exist among the eight species tested with *C. ocreatus*.

Taking all this into account, as well as the day when the first L-III appeared, and when all had reached this stage we can consider the most suitable species as being: *C. (C.) virgata*; *M. (A.) granulata*; *H. ordunensis*; *C. (X.) cespitum arigonis*; *C. barbara*. It must be pointed that although the degree of evolution and the percentage L-III were higher in *M. (A.) granulata*, larval development was slow.

In our opinion, *H. itala*, *O. (O.) barbula*, and *C. nemoralis* are not susceptible to *C. ocreatus*.

This is the first time that the susceptibility to *C. ocreatus* of *M. (A.) granulata*, *O. (O.) barbula* and *H. ordunensis* has been tested under experimental conditions, although the latter had been found together with *H. itala*, *C. (C.) virgata* and *C. (X.) cespitum arigonis* harbouring *C. ocreatus* in natural conditions by MANGA et al. (1979) and MANGA & MORRONDO (in print).

C. barbara, *C. (X.) cespitum arigonis* and *H. ordunensis*, species we consider favourable I.H. are amply distributed in the province of León (MANGA, 1977). This distribution concides with cattle-rearing areas and may be important in the epizootiology of this parasitosis.

REFERENCES

AZIMOV, D. A.; UBAIDULLAEV, Ya. V.; KULMAMATOV, E. N.; ZIMIN. Yu. M. & DADAEV, S. (1975).—(The life-cycle of nematode *Cystocaulus ocreatus* (RAILLIET & HENRY, 1907) in Uzbekistan). *Materialy Nauchnykh Konferentsii Vsesoyunozgo Obshchestva Gel'mintologov, 27:* 15-18.

CABARET, J. & DAKKAK, A. (1979).—Infestation expérimentale de *Cochlicella ventricosa* (DRAPARNAUD, 1801) par des larves L-I de Protostrongylides. *Annales de Parasitologie Humaine et Comparée, 54:* 57-64.

CORDERO, M. et al. (1980).—*Indice-catálogo de Zooparásitos Ibéricos.* Ministerio de Sanidad y Seguridad Social. Madrid.

DAVTIAN, E. A. (1940).—Cikl rasvitiya *Cystocaulus nigrescens. Trudi Armenii Nauchu-Issled Veterinaria Instituta, 3:* 5-31.

DAVTIAN, E. A. (1949).—Cikl rasvitiya nematod legkich ovest. i Koz Armenii *Zoologica Sbornik A.N. Armenii Nivi, 6:* 185-263.

EL-MOUKDAK, A. R. (1978).—Zur Entwicklung der Kleinen Lungenwürmer des Schafes in Laboratoriumstieren, mit einem Beitrag zur Differentialdiagnose der dritten Larven. *Zeitschrift für Parasitenkunde, 155:* 214-217.

GERICHTER, Ch. B. (1951).—Studies on the lung nematodes of sheep and goats in the Levant. *Parasitology, 41:* 166-183.

JOYEUX, C. & GAUD, J. (1946).—Recherches helminthologiques marocaines. Etude sur la pneuminie vermineuse. *Archives de l'Institut Pasteur du Maroc, 3:* 383-461.

MANGA, M. Y. (1977).—*Los Helicidae (Gastropoda, Pulmonata) de la provincia de León.* Tesis Doctoral. Facultad de Biología de León: 1-365 + 1-113.

MANGA, M. Y.; MORRONDO, M. P.; REGUERA, A. & CASTAÑON, L. (1979).—Datos sobre infestación natural de algunas *Cernuella* y *Helicella* spp. (Mollusca, Stylommatophora), por larvas de Protostrongylidae ovinos. *2.º Congreso Nacional de Parasitología*, León: 106.

MANGA, M. Y. & MORRONDO, M. P. (in print).—Notes of natural infection of some Helicidae spp. (Mollusca, Stylommatophora) by Protostrongylinae sheep larvae. *Malacologia.*

MORRONDO, M. P. & MANGA, M. Y. (in print).—Experimental study on the susceptibility of five Helicidae species to larvae of Protostrongylinae. *Malacologia.*

MORRONDO, M. P.; CORDERO, M.; DIEZ, P. & MANGA, M. Y. (en prensa).—Infestación experimental de tres *Cernuella* spp. (Mollusca, Stylommatophora) con larvas de *Muelleris capillaris* y *Neostrongylus linearis* (Nematoda, Protostrongylinae). *Anales de la Facutad de Veterinaria*, León, *26.*

RAMIREZ, A. P. (1967).—Epizootiologia de las bronconeumonias verminosas ovinas en León. *Anales de la Facultad de Veterinaria, León, 13:* 135-210.

TARAZONA, J. M. (1955).—Estudios sobre ciclos evolutivos y la terapéutica de las estrongilosis pulmonares ovinas. *Suplemento Científico del Consejo General de Colegios Veterinarios, 9:* 271-301 + 345-362.