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ORIGINAL PAPER

Arrested development of sheep strongyles: onset and resumption under field conditions of Central Europe

Iva Langrová · Kateřina Makovcová · Jaroslav Vadlejch · Ivana Jankovská · Miloslav Petrtýl · Jan Fechtner · Petr Keil · Andriy Lytvynets · Marie Borkovcová

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Abstract Two tracer tests were conducted between August 2004 and March 2007 at an ecological farm in western Bohemia. The first tracer test was performed for the summer–autumn grazing period (onset of arrested development), the second for spring (resumption of arrested development). In the first tracer test, the percentage of nematodes arresting development over the winter months reached 87.7% for *Teladorsagia circumcincta*, 66.7% for *Haemonchus contortus*, 89.9% for *Nematodirus filicollis*, 21.6% for *Trichostrongylus axei*, and 23.9% for both *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*. None of the arrested larvae were observed with species *Cooperia curticei, Nematodirus battus*, and *Oesophagostomum venulosum*. In the second tracer test, a significant increase of adult worms was discovered in March of species *T. circumcincta* and *N. filicollis* and *Trichostrongylus* spp. in February. Redundancy analysis and generalized linear models analyses have confirmed that environmental conditions play a crucial role in hypobiosis of sheep strongyles in the Czech Republic. The analysis of influences of various environmental factors revealed that the number of arrested larvae was negatively influenced by light—day length, sunshine, or daylight decrease ($p<0.01$).

I. Langrová (✉) · K. Makovcová · J. Vadlejch · I. Jankovská · M. Petrtýl · J. Fechtner
Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources,
Czech University of Life Sciences Prague,
Kamýcká 957,
165 21 Prague 6-Suchdol, Czech Republic
e-mail: langrova@af.czu.cz

P. Keil
Department of Ecology, Faculty of Science, Charles University,
Viničná 7,
128 00 Prague 2, Czech Republic

A. Lytvynets
Department of Laboratory Animals Breeding and Hygiene,
Institute of Physiology, Academy of Sciences of the Czech Republic,
Vídeňská 1083,
142 20 Prague 4, Czech Republic

M. Borkovcová
Department of Zoology, Fisheries, Hydrobiology and Apiculture,
Faculty of Agronomy, Mendel University of Agriculture and Forestry Brno,
Zemědělská 1,
613 00 Brno, Czech Republic

Introduction

Arrested development is a well-known phenomenon in some gastrointestinal nematodes of ruminants, particularly species of the superfamily Trichostrongyloidea. In gastrointestinal strongyles of domestic ruminants, the propensity for inhibition varies between and within species, depending on climate and management conditions. In some species of Trichostrongyloidea, the importance of climatic conditions has been well-recognized. In temperate regions, the nematodes usually inhibit development before the winter season. Winter inhibition is found in areas where transmission of infections occurs mainly in summer, and summer inhibition in areas where transmission is mainly from autumn to spring (Eysker 1993).

Many authors have often described the seasonal patterns of inhibited larval development of the two sheep nematodes *Haemonchus contortus* and *Teladorsagia circumcincta* from many parts of the world (Armour et al. 1966; Cleveland et al. 1968; Connan 1968; Reid and Armour 1972; Suarez and Busetti 1995; Uriarte et al. 2003; Waller et al. 2004). However, the reports about the seasonal arrest of development of the other sheep gastrointestinal strongyles are rare. An excellent review about the arrested development of explicit species or genera of nematodes was processed by Michel (1974).

The objectives of this project were to determine: (a) the strongyle species from all gastrointestinal tract of sheep undergoing the arrested development, (b) the month when strongyles cease their development as well as how long the arrested development by individual strongyle species lasted, and (c) which of the environmental factors most contributed to the arrested development in farm conditions. The main benefit of this project was the gaining of complex information about arrested development from all gastrointestinal tract of tracer sheep as well as the note about onset and resumption of this phenomenon.

Materials and methods

This study was conducted between August 2004 and March 2007 at an ecological farm in western Bohemia. The farm sheep (Oxford down, Suffolk) have never received any antiparasitical treatment. The flock comprised of 27 ewes and 23 lambs (6 months old). The flock of animals was grazed on a pasture of 2.25 ha as a permanent flock while the other lambs were used as tracer lambs. The pasture had not been grazed by other animals. Ewes and lambs remained on the pasture until snow cover, when they were removed to a barn. Weather data were recorded at a meteorological station located near the farm. The climate consists of characteristic temperate zone of Central Europe.

Two tracer tests were conducted in experimental periods. The first tracer test was performed for the summer–autumn grazing period (onset of arrested development), the second for spring (resumption of arrested development). For the tracer tests, lambs born in the same year were used, the tracer lambs had been rendered worm-free by anthelmintic treatment (Ivomec®, Merial).

The first tracer test (onset of arrested development) For three successive years, from August to December, two lambs were kept under worm-free conditions, and were allowed to graze for 4 weeks with the main flock. The two tracers were then housed on concrete for 2 weeks before being slaughtered.

The second tracer test (resumption of arrested development) This tracer test closely followed the first tracer test every year. In October 2004, 2005, and 2006, the six lambs kept under worm-free conditions were allowed to graze for 4 weeks with the main flock, and after this period, they were housed on concrete until their slaughters. Six tracers were slaughtered, two in January, two in February, and two in March.

Viscera (abomasum, small intestines, colon, and caecum) of all lambs were collected and processed for worm recovery, enumeration, and identification by the methods described by Eysker and Kooyman (1993). In general, 10–100% of the abomasal and intestinal contents, washing and digested material collected from each animal were examined by microscope (magnification ×100–×450).

The data were analysed using regression analysis (Cleveland et al. 1992). In all models effects, the sampling year and the type of tracer test (first or second) were used as covariates. Zero values were assigned weight 0.5 R^2 and the p levels stand only for the covariate-free effect of time. Environmental variables with the best potential to explain seasonal variability in nematode abundances (mean, maximum and minimum temperatures, the monthly rainfall, the amount of daily sunshine, the number of rainy days, the day

Table 1 Arrested development of strongyle species found in tracer lambs

Strongyle species	Adult nematodes		Nematode larvae		% Lambs with hyp. larvae	% Lambs with hyp. larvae in autumn[c]
	min[b]	max	min[b]	max		
Bunostomum trigonocephalum	1	72	3	3	2.1	0
Cooperia curticei	48	54	–	–	0	0
Haemonchus contortus	1	300	10	53	4.8	11.1
Chabertia ovina	1	829	9	99	8.3	5.6
Nematodirus battus	99	3713	–	0	0	0
Nematodirus filicollis	40	20120	5	7800	47.6	55.6
Oesophagostomum venulosum	1	81	–	–	0	0
Teladorsagia circumcincta	10	17296	9	5448	52.4	72.2
Trichostrongylus axei	23	20610	13	280	19.4	33.3
Trichostrongylus spp.[a]	82	22575	1	1554	30.1	55.6

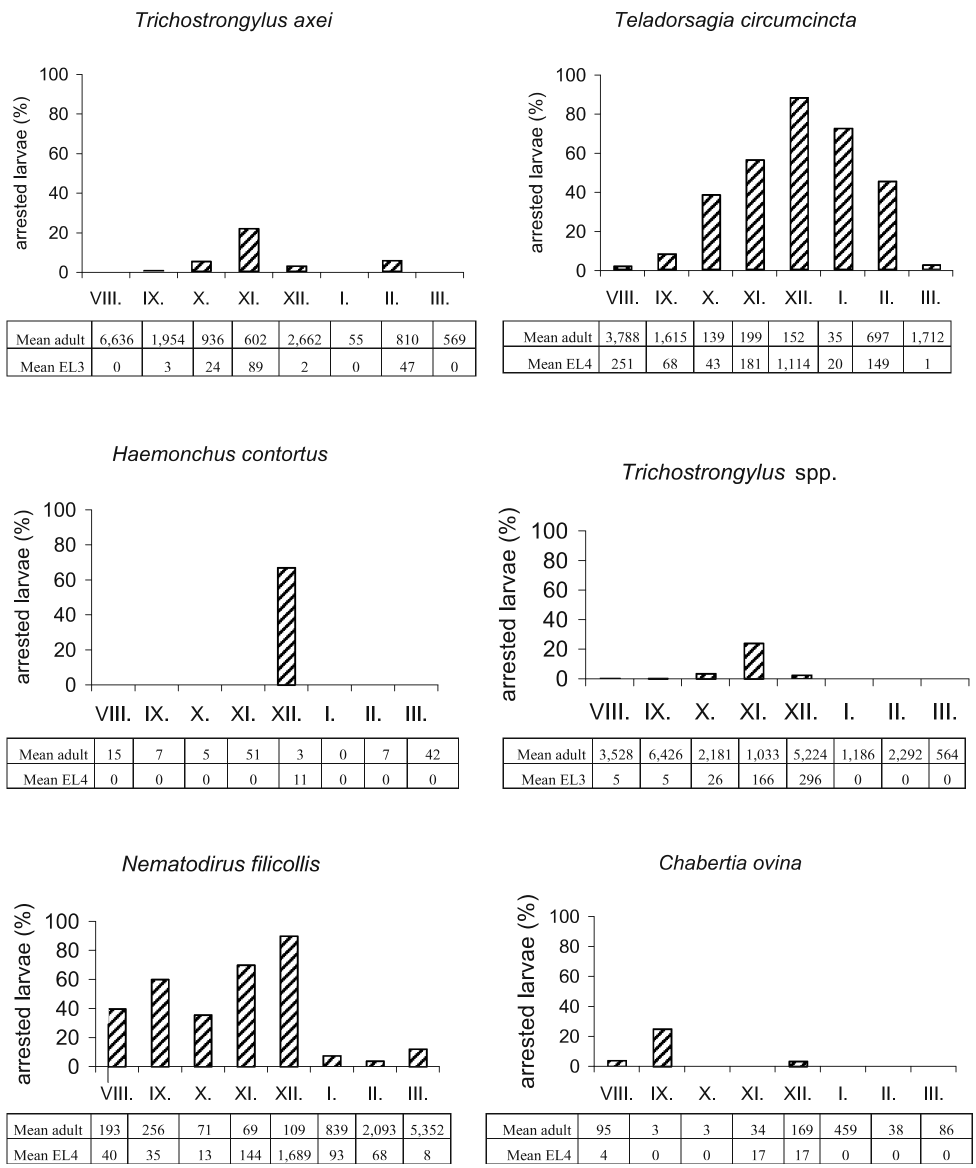
[a] *T. colubriformis* and *T. vitrinus*
[b] Cited only nonzero values
[c] Lambs pastured in October, November or December

lengths, and daylight decrease in month) were tested using the redundancy analysis (RDA) method (Lepš and Šmilauer 2003) and generalized linear models (GLM) (Crawley 2005) with log link function. RDA tested variables in the model (Monte Carlo permutation test, $F=3.53$, $p=0.002$). Generalized Linear Models were used to build a covariate model and the effect of all environmental variables on the covariate model was assessed using AIC (Akaike Information Criterion). Finally, the variable causing the highest decrease of AIC of the covariate model was selected.

Results and discussion

Out of 48 gastrointestinal tracts examined, all were found to be positive for nematode infection. Twelve species of gastrointestinal strongyles were recovered. *Trichostrongylus axei* was the most common nematode (42 animals positive, 87.5%), followed by *Trichostrongylus colubriformis* (75%), *T. circumcincta* (75%), *Chabertia ovina* (68.75%), *Trichostrongylus vitrinus* (62.50%), *Nematodirus filicollis* (52.08%), *Oesophagostomum venulosum* (50%), *Bunostomum trigonocephalum* (47.9%), *Nematodirus battus* (31.25%), *Haemonchus contortus* (29.17%), and *Cooperia curticei* (18.75%). *Ostertagia trifurcata* was found only once.

T. circumcincta was of the highest prevalence and intensity of arrested larvae (52.4% tracer lambs); *N. filicollis* (47.6%) and *Trichostrongylus* spp. (30.1%) followed (Table 1, Fig. 1.). The statistical evaluation is shown in Table 2. None of the arrested larvae were observed by *C. curticei, N. battus*, and *O. venulosum* species.



Trichostrongylus axei

	VIII.	IX.	X.	XI.	XII.	I.	II.	III.
Mean adult	6,636	1,954	936	602	2,662	55	810	569
Mean EL3	0	3	24	89	2	0	47	0

Teladorsagia circumcincta

	VIII.	IX.	X.	XI.	XII.	I.	II.	III.
Mean adult	3,788	1,615	139	199	152	35	697	1,712
Mean EL4	251	68	43	181	1,114	20	149	1

Haemonchus contortus

	VIII.	IX.	X.	XI.	XII.	I.	II.	III.
Mean adult	15	7	5	51	3	0	7	42
Mean EL4	0	0	0	0	11	0	0	0

Trichostrongylus spp.

	VIII.	IX.	X.	XI.	XII.	I.	II.	III.
Mean adult	3,528	6,426	2,181	1,033	5,224	1,186	2,292	564
Mean EL3	5	5	26	166	296	0	0	0

Nematodirus filicollis

	VIII.	IX.	X.	XI.	XII.	I.	II.	III.
Mean adult	193	256	71	69	109	839	2,093	5,352
Mean EL4	40	35	13	144	1,689	93	68	8

Chabertia ovina

	VIII.	IX.	X.	XI.	XII.	I.	II.	III.
Mean adult	95	3	3	34	169	459	38	86
Mean EL4	4	0	0	17	17	0	0	0

Fig. 1 Percentage share of arrested larvae from adults in specific nematodes during August-March 2004–2007. *Trichostrongylus* spp., *T. colubriformis* and *T. vitrinus*

Table 2 Environmental variables with best potential to explain seasonal variability in nematode abundances

Species	Stage	Variable with best fit	Explained deviance (%)	Effect	*p* value
Chabertia ovina	Adults	Sunshine	14.9	−	*
Oesophagostomum	Adults	Sunshine	14.7	+	*
Trichostrongylus axei	Adults	Rainy days	45.1	+	*
Teladorsagia circumcincta	Adults	Day length	52.8	+	*
Haemonchus contortus	Adults	Rainy days	10.2	+	*
Trichostrongylus colubriformis and *Trichostrongylus vitrinus*	Adults	Rainy days	20.6	−	*
Nematodirus filicollis	Adults	Rainy days	13.6	−	*
Chabertia ovina	Larvae	Day length	5.8	−	*
Oesophagostomum	Larvae				NS
Trichostrongylus axei	Larvae	Day length	13.8	−	*
Teladorsagia circumcincta	Larvae	Sunshine	15.2	−	*
Haemonchus contortus	Larvae				NS
T. colubriformis and *T. vitrinus*	Larvae	Maximal temperatures	48	−	*
Nematodirus filicollis	Larvae	Day decrease	71	−	*

NS Non-significant
*$p<0.01$

A number of authors described a seasonal pattern in burdens of arrested *H. contortus* as well as arrested *T.* (*Ostertagia*) *circumcincta* in sheep (Armour et al. 1966; Connan 1968, 1971; Blitz and Gibbs 1972; Reid and Armour 1972; Ayalew and Gibbs 1973; Uriarte et al. 2003; Waller et al. 2004). However, the seasonal arrested development of *N. filicollis* is rarely reported (Reid and Armour 1972; Ayalew et al. 1973). Similarly, there are few records about seasonal arrested development of *Trichostrongylus* genera. The authors like Herlich and Merkal (1963) and Eysker (1978) supposed that the immune status of host above all affect the arrested development of *Trichostrongylus* spp. However, Denham (1969) who infected immunized lambs with *T. colubriformis* found no arrested larvae. The statements from this study found out a seasonal trend in hypobiosis of *Trichostrongylus* spp., though the percentage of arrested larvae is not high. Similarly, the percentage of seasonal hypobiosis of *Trichostrongylus* spp. (20%) is shown also by Eysker (1978). Seasonal hypobiosis is reported also by Suarez and Busetti (1995) and Horak (2004). The authors of this study suppose that one of the main reasons there are so few reports on hypobiosis of *Trichostrongylus* spp. is the critical issue of methodology. These genera arrested in early L3 stage and exsheathed L3 larvae are very small (approximately 0.6 mm) and therefore can be easily overlooked. Therefore, the examination must be done only by microscope and with appropriate magnification.

In the second tracer test, a significant increase of adult worms was discovered in March in species *T. circumcincta* and *N. filicollis*, in February in *T. colubriformis*, *T. vitrinus*, and *T. axei* (Fig. 1).

The increase of fecal egg counts in spring months is frequently involved in phenomenon variously known as the spring rise, the postparturient rise, or the lactation rise. The worm eggs passed during the course of this spring rise play an important part in the epidemiology of strongyles. This phenomenon has come to be either so firmly linked with arrested development—the assumption being made that events associated with the loss of resistance, parturition, lactation, or seasonal influences trigger the development of arrested larvae—or also due to the uptake of new infection (the possibility overwintering infective larvae on pastures). Usually, the greatest importance is attached to the parturi-

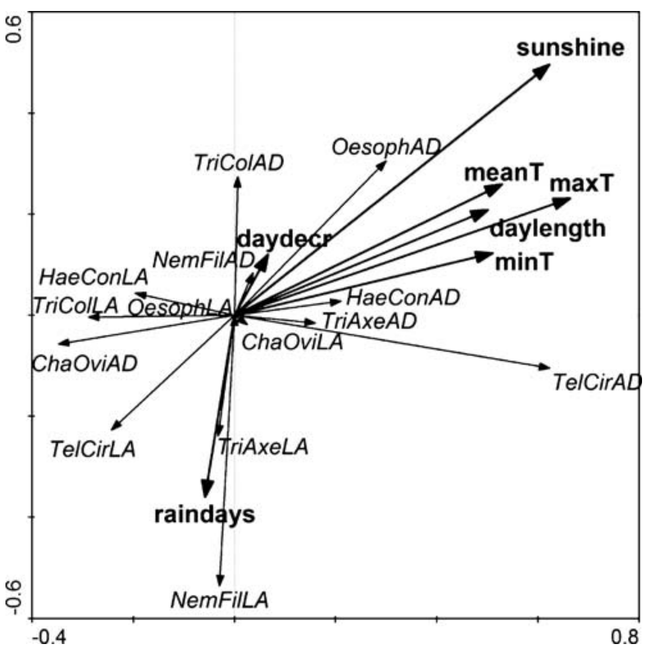


Fig. 2 The ordination diagram of the redundancy analysis (RDA) of nematode species composition. The ordination axes were constrained by all environmental variables (*TriAxe*, *Trichostrongylus axei*; *TriCol*, *Trichostrongylus colubriformis*; *Oesoph*, *Oesophagostomum*; *NemFil*, *Nematodirus fillicolis*; *HaeCon*, *Haemonchus contortus*; *ChaOvi*, *Chabertia ovina*; *AD*, adults; *LA*, larvae)

tion and lactation (Blitz and Gibbs 1971; Etter et al. 1999; Nǵangǵa et al. 2006). However, some authors describe the relevant seasonal factors (Field et al. 1960; Brunsdon 1964; Gibbs and Barger 1986). Gibbs and Barger (1986) supposed that the trigger for stimulation of the rise may not have been related to the changes associated with parturition and lactation but it could have been a seasonal stimulus derived from within larvae. Nǵanǵa et al. (2006) interpreted the highest nematode egg output in time, when lambing of ewes coincided with the end of the dry season, as result of maturation of hypobiotic larvae.

The spring rise is probably a phenomenon caused by many factors and is very difficult to explain. Different triggers can act in various intensity, separately or in all, in various climate zones and in various management conditions. We suppose that the spring rise is primarily seasonally influenced, and at the same time this phenomenon is intensified by the lactation or pregnancy of ewes.

One of the main objectives of the study was to ascertain which environmental factors are necessary for the induction of strongyle hypobiosis. The RDA analysis established a significant gradient of temperature and available light along the first horizontal axis (0.088, F=4.012, p=0.016) clearly separating adults and larvae (Fig. 2). The GLM analysis revealed that the number of larvae was negatively influenced by the light—the day length, sunshine or daylight decrease (Table 2). Therefore, the RDA and GLM analyses confirm that the environmental conditions have the critical role in the hypobiosis of sheep strongyles. For the species of *T. circumcincta* and *N. filicollis* seasonal affection is with great probability, crucial. The seasonal conditions are also important for *Trichostrongylus* spp.

In previous years, it was established that in temperate climates hypobiosis seems to be connected to the advent of changing photoperiod and cool temperatures. The infective larvae, that are exposed to environmental stimuli, particularly low temperature, will inhibit development (Armour and Bruce 1974; Michel et al. 1974, 1975; Watkins and Fernando 1984). Currently there are findings that suggest not only low temperatures but also photoperiod contributed to the arrested development of many nematode species. Clearly it is related to the diapause phenomenon, and consequently the phylogenetic relationship of nematodes to arthropods (the diapause phenomenon is triggered primarily by the photoperiod).

Our analysis of the influence of various environmental factors on the onset of arrested development revealed the relevance of the photoperiod. In the Czech Republic, like in the northern hemisphere, the photoperiod decreased in the autumn–winter season, and we supposed that these changes act as one of the main precursor signals to the infective larvae of strongyles to stop their development in the hosts at an early parasitic larval stage and these then wait until the opportunity for transmission to another host is available. The analogous findings to this study were reported by Gibbs (1973), Armour (1978), Fernández et al. (1999), Langrová and Jankovská (2004), Lützelschwab et al. (2005).

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