Irrigation, faecal water content and development rate of free-living stages of sheep trichostrongyles

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Original article

Irrigation, faecal water content and development rate of free-living stages of sheep trichostrongyles

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Summary — The effect of irrigation by flooding the pastures on the ability of the eggs of sheep Trichostrongyles to develop into infective larvae (L3) was estimated in outdoor conditions by depositing parasitised faeces on plots, without irrigation or submerged, at different times and durations. The rates of development of *Teladorsagia circumcincta*, *Trichostrongylus vitrinus* and *Chabertia ovina* were very low in spring and summer in dry plots but proportional to the duration of submersion in the irrigated plots. *T circumcincta* was mainly favoured in spring, *T vitrinus* in summer, but higher rates were observed in autumn. The action of water had more effect on freshly deposited faeces.
In laboratory experiments on *T circumcincta*, *T vitrinus* and *Haemonchus contortus*, the submersion of cultures was unfavourable to egg development but favoured L_1 development. Cultures with different faecal water contents (FWC) simulating a submersion (7 or 16 h) or an alternance of spraying and dehydration, or at constant values of 60 and 50% enabled us to conclude that high FWC favoured the development of *T vitrinus*, had some negative effect on *T circumcincta*, but *H contortus* was more susceptible to variations of FWC. The FWC had an effect on the size of L_3 and, for *T circumcincta* only, the survival rate of the small L_3 obtained at low FWC seemed decreased. These data could explain the absence of *H contortus*, as well as the abundance of *T vitrinus* on the studied pastures.

development / irrigation / sheep / Trichostrongyle / larvae

Résumé — Irrigation, teneur en eau des fèces et développement des stades libres des trichostrongles d'ovins. *L'effet de l'inondation des pâturages sur les possibilités de développement des œufs de strongles d'ovins en larves infestantes (L3) a été étudié dans des conditions naturelles par des dépôts de fèces parasitées sur des parcelles non irriguées ou immergées à des moments et pendant des durées variables. Les taux de développement de* Teladorsagia circumcincta, *de* Trichostrongylus vitrinus *et de* Chabertia ovina *ont été très faibles sur les parcelles sèches au printemps et en été, et proportionnels à la durée de l'immersion sur les autres.* T circumcincta *était fa-*

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vorisé au printemps, T vitrinus *en été. C'est en automne que les taux maximaux ont été observés. L'action de l'eau était d'autant plus favorable que l'irrigation avait lieu sur les œufs et les L1 plutôt que sur les L2 ou les L3. Au laboratoire, l'immersion de cultures de* T circumcincta, *de* T vitrinus *et d'*Haemonchus contortus *avait un effet défavorable si elle avait lieu sur les strongles au stade œuf, favorable au stade L1. Des cultures à diverses conditions de teneur en eau des fèces (TEF) simulant une immersion (de 7 ou 16 h), une alternance d'aspersion et de déshydratation, ou à des valeurs constantes de 60 ou 50%, ont permis de montrer que les TEF élevées favorisaient* T vitrinus, *avaient quelques effets défavorables sur* T circumcincta, *et que* H contortus *était particulièrement sensible aux variations de TEF. De plus, la TEF influait sur la taille des L3, mais non sur leur survie. Ces données contribuent à expliquer l'absence d'*H contortus *et l'abondance de* T vitrinus *sur les pâturages étudiés.*

développement / irrigation / ovin / nématode parasite / larve

INTRODUCTION

Freshly deposited faecal pellets of small ruminants are submitted to environmental conditions that influence the development of trichostrongyle eggs. Conditions at deposit time are important (Dinaburg, 1944), and desiccation is responsible for the high mortality of the eggs and pre-infective larvae (Mauleon and Gruner, 1984; Uriarte *et al*, 1984; Berbigier *et al*, 1990). Irrigation of pasture favours the development of the free-living populations by decreasing soil temperature and increasing humidity (Bullick and Andersen, 1978; Gruner *et al*, 1989; Uriarte and Gruner, 1989).

In the south of France, some sheep pastures are irrigated by flooding, and a high level of infection is observed in sheep with diversified strongyle communities (Gruner and Cabaret, 1985).

The purpose of this study was to measure the effects of irrigation on the development of the main species of strongyles. In outdoor experiments, faeces deposits were done on dry and on flooded plots. In laboratory conditions, the specific effect of variations of faeces water content was investigated on the development rate of eggs into infective larvae of three species and on the survival of these larvae.

MATERIALS AND METHODS

Outdoor conditions

Two experiments were conducted at 'Domaine du Merle' (Salon de Provence), in the southern part of France with a humid Mediterranean climate (annual rainfall 640 mm). The soil is composed of an upper part of mud deposited by floods onto a calcareous substratum. The objective of the first experiment was to measure the development rate of eggs into infective larvae in faeces deposited on dry or irrigated pasture, immersion taking place at the end of larval development. In the second experiment, the flood took place at different stages of the development. In Experiment 1, fresh faeces were collected from a naturally infected flock, homogenized and deposited on 4 plots. Plot A was dry without any irrigation, the soil water content (SWC) remaining between 3 and 6%. Plot B received 2 cm of water for 4 h; then the SWC decreased from 29 to 25% at d 3 and 24% at d 7. Plot B was flooded for 12 h. Here, the SWC quickly decreased from 33% to 24% at d 3 and 21% at d 7. Plot D was flooded deeply (> 5 cm of water) for 14 h and the SWC decreased from 32% to 26% at d 3 and 24% at d 7. Apart from changes in the soil water content, the effect of irrigation was to decrease maximum soil temperatures, and to increase the time during which the relative humidity of the air near the soil was saturated (from zero in plot A to 13–16 h/d in plots B, C and D). In each plot, 100 g of faeces were deposited on the pasture surface inside 10 PVC plastic tubes (16 cm in diameter, 20 cm-long) half-sunk into the soil. Control cultures of faeces were conducted in laboratory conditions for 2 wk at 20–22°C. The species composition of the deposits was *Teladorsagia circumcincta* (74%), *Trichostrongylus vitrinus* (17%) and *Chabertia ovina* (9%). Faeces were deposited on 3 occasions during the irrigation season: on 5th June (spring), 11th August (summer) and 26th September (autumn). Plots B, C and D were flooded 14 d post deposit; 5 tubes were collected on d 13 (d 14 in September) and on d 20–22 (1 wk after the irrigation time). Infective larvae were separately extracted from faeces, grass, and the top 5 cm of soil by baermannization. In Experiment 2, 20 tubes were prepared in Plot C and faeces deposits were carried out at 4 different times, on d 10, 3 and 1 before flooding and on the flooding day (31st August). At irrigation time, the development stages were respectively L_3, L_2, L_1-EE (embryonated eggs) and eggs. Faeces, grass and soil were collected 14 d after deposit.

Laboratory conditions

Experiments were conducted separately with 3 species of gastro-intestinal nematodes, *T circumcincta*, *T vitrinus* and *Haemonchus contortus*. Each species was produced in lambs kept in a digestibility box. Faeces < 24 h were collected; the density of eggs was estimated by faecal egg counts. Faecal water content (FWC) was measured by weighing before and after drying 20 g 24 h at 100°C.

Experiment 3 was conducted to study the effect of the temperature of the water during the 16 hours of immersion and of the stage of development at the time of immersion (morula, EE, L_1, L_2 and L_3). Ten rectangular plastic flasks (120 ml) containing 10 g of faeces per condition were used. To obtain the different stages, faeces were cultured at 26°C respectively 0–2 h, 8 h, 24 h, 72 h and 170 h for *T circumcincta* and *T vitrinus* and 0–2 h, 6 h, 23 h, 72 h and 144 h for *H contortus*. At the end of immersion, excess water was separated, contained larvae were counted, and the faeces were placed in Petri dishes again at 26°C until the 9th d (8th d for *H contortus*). Control cultures without immersion were placed in similar conditions.

Experiment 4 was conducted to study the effect of different kinetics of faecal water content simulating absence or presence of irrigation by immersion or aspersion on the rate of development. Individual cultures of 10 g of faeces were conducted at 20 ± 1°C in 100 cm^3 boxes under 5 FWC conditions, with 15 replications per condition. Condition A (control one) was a constant FWC of 60% (which corresponds to a water content of freshly deposited faeces), every box being weighed 1 to 3 times per day. Condition B was a drier and constant FWC (50% on average). Fresh faeces were covered with water for 7 h (condition C) or 16 h (D), then dried with an air current until the water content level decreased to about 60%. Condition E was a daily humidification simulating irrigation by sprinkling, in which 2.5 g water was added to each culture, this during the first 4 days. The FWC of conditions C, D and E were averaged during the total time of the culture to a value close to 60% by adding some water or keeping boxes uncovered to diminish the FWC. For each culture, maximal, minimal and mean values for FWC were estimated for culturing d 1, 2, 3 and 4, and for the periods 1–2 d, 1–3 d, 1–4 d and total. The cultures were baermannized for larval counts after 11 d for *H contortus* and 14 d for the other species. The length of *T circumcincta* and *T vitrinus* larvae from each experiment was measured by using an Analytical Measurement System VIDS III (Shirehill Industrial Estate, Saffron, Walden, UK). The survival of larvae in each of the experimental conditions was estimated in water at 20 ± 1°C every 1 to 2 months by counting active larvae after touching them with a needle, to see if they were alive.

RESULTS

Development in outdoor conditions

The development of eggs into larvae in faeces deposited in dry or irrigated pasture (table I) was higher in autumn for the 3 species when compared with spring and summer deposits. In spring, higher levels of *T circumcincta* were observed 20 d post-deposit in the irrigated plots B, C and D. In summer, higher development of *T vitrinus* occurred at d 13 on irrigated plots. On d 20, a week after the flooding, practi-

Table I. Rates of development of strongyle eggs deposited within faeces in plots A, B, C and D respectively flooded 0, 4, 12 and 14 h (Experiment 1), expressed as the ratio of L_3 to L_3 in control cultures (means of 5 replications).

Removal (d)	T circumcincta				T vitrinus				C ovina			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Spring deposits												
13	0	0.02	0.01	0.02	0	0.01	0	0.01	0	0.03	0.01	0.06
20	0	0.36	0.14	0.81	0	0.08	0.05	0.14	0	0.04	0.01	0
Summer deposits												
13	0	0	0.01	0.03	0	0.16	0.05	0.15	0	0.02	0.04	0.06
20	0	0	0	0	0	0.02	0.04	0.03	0	0.01	0.01	0.01
Autumn deposits												
14	0.02	0.08	0.12	0.13	0.03	0.04	0.10	0.07	0.02	0.14	0.23	0.16
20	0.35	0.35	0.39	0.51	0.60	0.58	0.61	0.52	0.17	0.16	0.56	0.85

cally no larvae were observed in the faeces, grass and superficial soil. Between the plots, higher development rates were respectively in D, C and B, then in the non irrigated plot A, in accordance with the time of immersion. The comparison of the effect of irrigation on the different stages (table II) indicated that the influence of irrigation was more evident on the eggs and young larvae. The rates of development (L_3/100 deposited eggs) were low in this experiment carried out in the summer.

Development in laboratory conditions

In experiment 3 (table III), a negative effect was observed when the irrigation occurred on egg and L_2 stages. This effect was more severe at 26°C (and at 15°C for *T circumcincta*) compared with 18–20°C. For *T vitrinus*, the number of L_3 obtained after the immersion at the L_1 stage was more than twice the number obtained in the control cultures. Similar results were observed for *T circumcincta* when immersion occurred on faeces containing the first L_3.

In experiment 4, the rate of development of *T circumcincta* (table IV) was lower in conditions B (dry), than in C and D (faeces immersed for 7 or 16 h in water and then dried). *T vitrinus* was less affected by the different conditions. For these two species, the size of L_3 were shorter in condition B than in more humid conditions. *H contortus* was more affected by the lower humidity in B than by the variations of faecal water content with spraying and desiccation in E. The calculation of the kinetics of the FWC day by day for the 75 samples (15 replicates in 5 conditions) explained the variability of the number of larvae by a stepwise regression analysis: 63% of the variability was explained by 4 variables, minimal FWC at d 3, mean FWC at d 2 (with positive coefficients), minimal FWC at d 2 and d 4 (with negative coefficients).

Table II. Comparison of the suceptibility to flooding between the stages of sheep trichostrongyles in Experiment 2 (means of 5 replications).

	Time faeces deposit-irrigation			
	0 d	*1 d*	*3 d*	*10 d*
Stage at irrigation time	*Egg*	L_1	L_2	L_3
Irrigation-recovery delay	*14 d*	*13 d*	*11 d*	*4 d*
Total L_3/L_3 in control	0.26[a]	0.27[a]	0.18[b]	0.14[b]
T circumcincta	0.28	0.33	0.24	0.13
T vitrinus	0.37	0.40	0.28	0.16
C ovina	0.08	0.14	0.11	0.02
Total L_3/100 deposed eggs	4.7	4.7	3.1	1.3

Significant differences ($P < 0.05$) between [a] and [b].

The survival at a constant temperature of 20°C of the L_3 obtained from these cultures (fig 1) presented a rapid decrease during the 4th month of storage for *T vitrinus* and between 6–8 months for *H contortus*. A low decrease between 5–8 months was recorded for *T circumcincta*; variations between culture conditions were larger than those established in the 2 other species, with a higher mortality of L_3 issued from the cultures with lower FWC (fig 1).

Table III. Effect of 16 h immersion of the faeces in water at different temperatures on the development of the different stages of *T circumcincta*, *T vitrinus* and *H contortus*.

	T circumcincta			T vitrinus		H contortus	
Eggs/10 g faeces		*585 ± 25*		*604 ± 24*		*9014 ± 205*	
	15°C	*20°C*	*26°C*	*18°C*	*26°C*	*18°C*	*26°C*
Morula	0.19	0.32	0.24	0.24	0.09	0.04	0.03
Embryonated egg	0.28	0.94	0.48	0.25	0.17	0.43	0.08
Larvae 1	0.60	0.68	0.09	2.75	2.33	1.89	0.98
Larvae 2	0.17	0.43	0.17	0.32	0.27	0.48	0.37
Larvae 3	1.96	2.73	1.07	1.08	0.93	0.83	0.81

The larvae were collected respectively after 9 d, 9 d and 8 d at 26°C and expressed as the ratio to L_3 from non immersed control cultures (means of 10 replications).

Table IV. Development of free-living stages of *T circumcincta*, *T vitrinus* and *H contortus* at 20°C in faeces submitted to different faecal water content (FWC) during Experiment 4.

		Conditions				
		A Control	*B Dry*	*C 7 h in water*	*D 16 h in water*	*E Aspersed + dried*
FWC (%)	*Mean*	*59*	*53*	*62*	*62*	*61*
	range	*51–65*	*50–59*	*57–82*	*54–82*	*57–70*
T circumcincta						
L_3/10 g	Mean (v)	9127 (0.14)	5441 (0.15)	4564 (0.29)	7023 (0.24)	8649 (0.25)
L_3 length (μm)		828	784	799	792	837
T vitrinus						
L_3/10 g	Mean (v)	1105 (0.25)	1035 (0.25)	1096 (0.25)	1291 (0.21)	1145 (0.19)
L_3 length (μm)		710	703	718	719	738
H contortus						
L_3/10 g	Mean (v)	516 (0.25)	169 (0.25)	353 (0.25)	359 (0.21)	229 (0.19)

Means of 15 replicates of cultures of 10 g of faeces and (v) = standard deviation/mean.

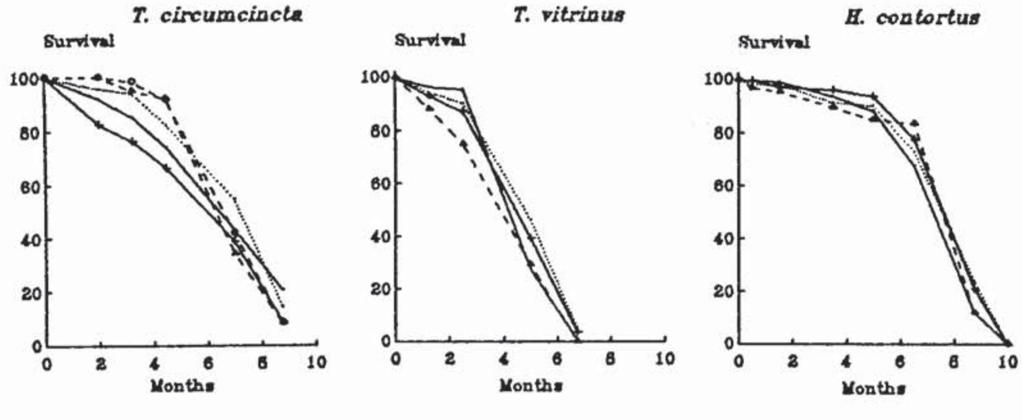

Fig 1. Survival (%) in water at 20 ± 1°C of the L3 issued from cultures with different faecal water content (FWC) of experiment 4: constant FWC of 60% (•—•) or near 50% (+—+), faeces sunk 7 h (Δ—Δ) or 16 h (O—O) then dried, or submitted to an alternance of spraying and desiccation (●······●).

DISCUSSION

In outdoor experiments, the flooding of the pastures increased the air humidity and lowered the maximal temperatures of the soil as observed by Andersen *et al* (1974). Free-living stages of nematode parasites were then able to develop. The effect of deposit conditions was expressed by comparison with larvae from control cultures. No development was observed in the non-irrigated plot in spring or in summer; Callinan (1978) had similar results in the dry mediterranean conditions of Australia and Uriarte *et al* (1984) in Spain. Maximal development occurred in the most flooded plot, mainly for *T circumcincta* and *C ovina*. The first species was more affected during the summer (table I). The positive effect of irrigation was most efficient if flooding occurred early in the development, at the egg or L_1 stage (Experiment 2, table II). This experiment was conducted during the summer, so faeces were submitted to desiccation until irrigation took place. Uriarte and Gruner (1989) found higher rates of development on plots irrigated by spraying in Spain during the summer for *T circumcincta*, *T colubriformis* and *H contortus*. Curiously, this last species was not found in the studied pastures, so that investigations were initiated in laboratory conditions to try to understand the reasons for its absence. Was it due to the fact that pastures were flooded?

Laboratory experiments were conducted to investigate the different factors relative to irrigation that could influence the rate of development. In practice, the temperature of the water used to flood the pastures was low, compared with that of the ambient air (12–15 *vs* 25–29°C during summer). So we wanted to separate the effect of variation of temperature from that of immersion. Some negative effect of low temperatures was registered on eggs of *T circumcincta* and *H contortus*, and of high temperatures (26°C) on eggs of *T vitrinus* and *H contortus* (table III). Eggs were more susceptible to the negative effect of immersion than the other stages. If we compared the rate of development of the 3 species (Experiment 4), *T vitrinus* development was favoured by high values of faecal water content (FWC); *H contortus* was affected by low FWC and by the alternance of high and low values of FWC. *T circumcincta* was less affected. The observed higher susceptibility of *H contortus* to immersion could contribute to explaining the absence of this species in the flooded plots. In all cases, it was the conditions during the first days of development that were more important, as Dinaburg (1944) and Andersen and Levine (1968) observed. The survival of the L_3 in water at constant conditions of 20°C was quite long, 4 months for *T vitrinus* and 6–8 months for the other species. Stewart and Douglas (1938) found that *Trichostrongylus axei* survived for 7 months in similar conditions at 5 mm depth and at ambient temperature. Survival decreased with depth as Tripathi (1969) observed for *H contortus* and *Trichostrongylus* sp (respectively, from 40 and 85 d at 1 cm to 30 and 50 d at 8 cm depth).

In conclusion, the irrigation of pastures by flooding favoured the development of freshly deposited eggs of sheep Trichostrongyles. With faeces immersion, a number of L_1 were liberated from the faeces as the L_3 were taken from faeces into the soil by water (Suryahadi and Gruner, 1985). The high values of FWC favouring *T vitrinus*, have some negative effects on *T circumcincta*, but *H contortus* was more susceptible to desiccation and variations of FWC. The conditions of FWC influenced the rate of development but also the size of the infective larvae and possibly their survival in some cases, as we observed for *T circumcincta*.

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