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Original Contribution

EFFECTS OF TEMPERATURE AND DESICCATION ON SURVIVAL RATE OF *HAEMONCHUS CONTORTUS* INFECTIVE LARVAL STAGE

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ABSTRACT

Small ruminants are among the most commonly farmed livestock in Bulgaria. The climate and geographic conditions and pastoral way of growing lead to higher infestation of herds by variety of soil-transmitted helminths. The most spread nematodes are gastro-intestinal strongylids (GIS) causing various diseases of sheep and goats. Abomasal parasite *Haemonchus contortus* (Nematoda, Trichostrongylidae) constitutes a major part of gastro-intestinal helminthic fauna of small ruminants and is one of the most pathogenic members of this family causing serious economic losses of sheep and goat farms. Pre-parasitic period of *H. contortus* consisting of segmented eggs, non-infective larvae first and second stage (L1, L2) and infective larvae (L3). This development is also known as exogenous phase and takes place only in environment. Presence of viable and infective L3 on pastures is fully depended on climate conditions, solar radiation, grass and soil type etc. However, temperature and humidity are the most important factors exerting a marked effect on survival of *H. contortus* L3 on pastures. Resistance of L3 to some atmospheric variables e.g. temperature and humidity could be used to predict occurrence of haemonchosis among small ruminants. The present study aimed to investigate the effect of temperature and desiccation on L3 vitality of *H. contortus* under laboratory conditions. Experimental infection in lambs by *H. contortus* was reproduced. Feces were collected after beginning of the patent period of infection. Fecal samples were cultivated for 10 days at 27ºC for developing infective L3 which were then obtained by routine Baermann technique. Acquired L3 were placed under various temperatures (-4ºC; -18ºC; 40ºC; 45ºC; 50ºC) and desiccation. The results clearly showed that L3 were more resistant to desiccation, -4º and 40ºC and less to -18ºC, 45ºC and 50ºC.

Key words: *Haemonchus contortus*, infective larvae, desiccation, temperature

INTRODUCTION

Vitality and infectivity of strongylid L3 in environment depends on cumulative effect of the three main atmospheric variables, namely temperature, humidity and ultraviolet (solar) radiation, which directly influence on larvae survival rate and therefore regulate their bioavailability of pastures. The presence of protective sheath and ability of larvae to perform horizontal and vertical migration represent a kind of adaptative mechanism limiting somewhat the harmful effects of unfavorable climate factors (1). According to Todd et al. (2), only 1% of infective *H. contortus* larvae survive more than 24 hours at -10ºC. In a similar experiment, Jasmer et al. (3) evaluate the effect of freezing (at -10ºC for 15 hours) followed by incubation (3ºC for 9 hours) on the vitality level of *H. contortus* infective larvae. The authors found no living L3 on day 14 after the temperature cycle. Gruner et al. (4) affirmed that the larvae retain vital for 300 days at 20ºC, and the highest survival rate (95%) is observed between 150th and 180th day. The results of similar studies revealed that over 90% of *H. contortus* larvae survive for 10 weeks at 3ºC (3, 5).

Vital *H. contortus* L3 were isolated from pasture on day 93 at 12ºC and at high relative humidity (5). Data presented in the same investigation also showed a significant larval reduction on day 9 at 28^{0}C and 35% relative humidity. Similar results were also obtained by Carneiro et al. (6) who reported that *H. contortus* larvae survive on pastures for several weeks in average monthly temperatures of around 17ºC. Zajac (7) and Morgan et al. (8)

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summarized the data on studies performed earlier and concluded the increasing temperature leads to reduction of L3 both in environment and under laboratory conditions. The authors assumed the presence of cuticle layer inhibits an access of nutritive elements to the larva and hence L3 survival is fully depended on its own energy reserve stored into the intestine cells. Heat accelerates larval metabolic processes which resulting on faster depletion of available nutrients and decreasing survival rate. In this relation, Todd et al. (2) found only 7% alive *H. contortus* L3 at 45ºC for two days. Protective sheath defends the larvae not only from unfavorable temperature but also from desiccation (5, 9). However, repeated dehydration affects *Trichostrongylus colubriformis* larvae worse than single influence (10). In contrast, Lettini et al. (11) affirmed that the process of drying followed by rehydration (known as anhydrobiosis) does not cause significant injury of infective larvae. Furthermore, the survival rate is extended due to reduction of metabolic activity. However, multiple and prolonged anhydrobiosis reduces the vitality of *H. contortus* L3 (12).

MATERIALS AND METHODS

Infective larvae were acquired from experimentally infected with *H. contortus* lambs. Prior to the study, an approval for using animals in experiment was obtained from the Bulgarian Food Safety Agency (permit No. 107). Feces were collected after beginning the patent period of infection. Fecal samples were cultivated for 10 days at 27ºC for developing of infective L3 which were then obtained by routine Baermann technique. Acquired infective larvae were exposed to different variation of temperature and desiccation **(Table 1).** Key criteria in assessing the harmful effects were changes in the larval morphology and behavior such as paleness and destruction of intestinal cells, bubble inclusions in larval body, flexions or corrugation onto the larva and changes in motility.

Table 1. *Influence of temperature and desiccation on L3 of H. contortus*

	Influence
1	Freezing at -4ºC and -18ºC for 12, 24, 48, 72 and 96 hours followed by cultivation at 27ºC for 6 hours
2	Double-freezing at -18ºC for 12 hours each followed by cultivation at 27ºC for 6 hours
3	3-fold freezing at -4ºC for 12 hours each followed by cultivation at 27ºC for 6 hours
4	Storage at 4ºC for 15 months
5	Heating at 40º C, 45 and 50 for 15 days, 2 days and 24 hours, respectively
6	Drying at room temperature from 1 to 12 hours followed by hydration
7	6-fold drying for 1 hour each at room temperature followed by hydration

RESULTS

Temperature of 40ºC negatively correlated with the survival rates of *H. contortus* L3. In addition, the same temperature value did not significantly affect the larvae vitality and motility during the first 3 days (**Table 2**). Prolonged heating also induced an exsheating of larvae (**Figure 1**) which were paler and the border between intestinal cells was unclear **(Figure 2)**. Decreased motility was observed on day 10 after heating. No mobile larvae were detected on day 14 and 15. Heating of L3 at higher temperatures (45ºC and 50ºC) caused 100% lethality as early on hour 48 and 24 of exposure, respectively. Data obtained by influence of low temperatures on L3 vitality are presented in **Table 3**. Only 18.1% of L3 were alive after storing at 4ºC for 15 months. Immotile larvae were filled with bubbles over the entire body length and the intestinal cells were fully degenerated **(Figure 3**).

The single drying did not adversely affect L3 until 3rd hours at the beginning of experiment and more than half L3 retain the active movements. A sharp decline in number of motile larvae was observed at 4th hour. The most of L3 were exsheated and broken in places was also presented. Twice drying stored the most larvae (72.2%) and differences compared to single influence were negligible (2.8%). Larvae were actively motile and time needed for motility reversion was significantly short (a few seconds). Later, L3 slowly regained their mobility but integrity of intestinal cells was impaired (**Table 4)**.

***Table 2**. Effect of 40^{0}C and duration period (DP) on vitality of H. contortus L3*

DP (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
% Vital L3	100 %	100 %	93 %	67.7 %	56.9 %	37.9 %	30 %	28.7 %	24 %	23.4 %	19.7 %	14.1 %	12.3 %	---	---

Figure 1. Protective sheaths of *H. contortus* infective larvae after heating

Figure 2. Exsheathed *H. contortus* L3 after heating

***Table 3**. Influence of low temperatures on vitality of H. contortus L3*

LT	**Exposure (hours)**	**Times**	**% vital L3**
-18^{0}C	12 hours each	Twice	5.7%

	12	Once	5.7%
	24		2.7%
	48		2.2%
	72		---
	96		---
-4^{0}C	12 hours each	Three-fold	28.6 %
			23.5%

	12	Once	28.6%
	24		4.8%
	48		3.3%
	72		---
	96		---

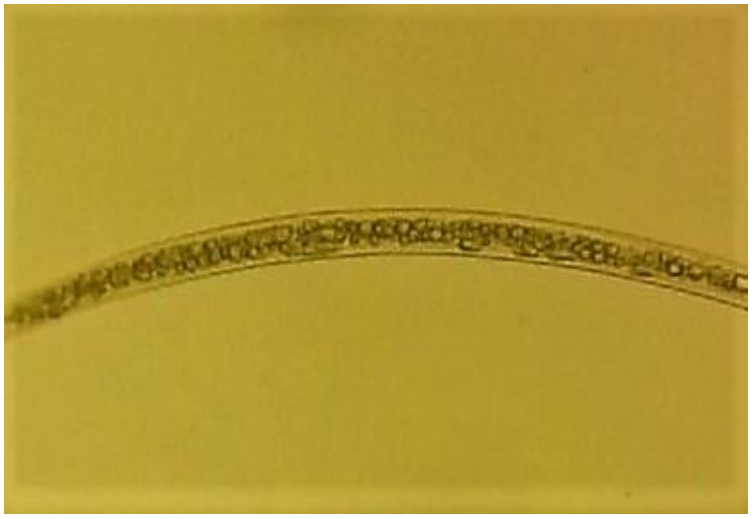

Figure 3. Bubble degeneration of *H. contortus* L3 after prolonged storage

Table 4*. Influence of desiccation on vitality of H. contortus L3*

Duration (hour)	1	2	3	4	5	6
% vital L3	79.5%	75%	53.6%	30%	11.8%	10%
Times	1	2	3	4	5	6
% vital L3	75%	72.2%	30.6%	2.1%	--	--

DISCUSSION

Our results showed that temperatures above 40ºC significantly influence larvae mortality. It is in agreement with data of Anderson et al. (13) who affirmed the infective larvae of *Trichostrongylus* spp. quickly die at 45ºC and 50ºC. In similar investigation Todd et al. (2) also reported a high mortality of *H. contortus* L3 at 45ºC for 2 days while only 7% remained alive. Zajac (7) and Morgan et al. (8) also found that increasing temperature reduced the survival rate of strongylid larvae. The authors assumed that the presence of sheath inhibits an access of nutritive elements to the larva and thus L3 survival is fully depended on its own energy reserve stored into the intestine cells. Heat accelerates larval metabolic processes which resulting on faster depletion of available nutrients and decreasing survival rate. It is well known that the enzyme system operates under strictly defined upper and lower temperature limits. Higher temperature levels cause denaturation of the enzymes which are also proteine molecules and lead to a collapse on the metabolic processes.

Our data also demonstrated the freezing at -18ºC greatly reduce the larval vitality and only 2.7% of L3 survived for 24 hours. Similar results were presented by Todd et al. (2) who reported that only 1% of *H. contortus* L3 remained alive at -10ºC for 24 hours. According to Andersen et al. (13), L3 of *Trichostrongylus colubriformis* survived for 8 days at the same temperature. The results presented in this study are also in agreement to published by Jasmer et al. (3) and Pandey et al. (14) who compared the temperature resistance rate between L3 of *H. contortus* and *Teladorsagia circumcincta*.

Our data demonstrated that only 18.1% of L3 were alive after storing at 4ºC for 15 months. This fully coincide with results of Andersen et al. (13) who revealed that L3 of *Trichostrongylus* spp. survived 425 days at 4ºC. In this relation, over 90% of *H. contortus* L3 remained alive at 3ºC for 10 weeks (3, 5).

Relatively high resistance of L3 to variations of humidity was attributed to the presence of additional cuticle layer functioning as biological shield protecting larvae from drying out (5, 9). However, repeated dehydration affects *Trichostrongylus colubriformis* L3 worse than single influence (10). In contrast, Lettini et al. (11) affirmed that anhydrobiosis does not cause significant injury of infective larvae. Furthermore, the survival rate is extended due to reduction of metabolic activity. However, multiple and prolonged anhydrobiosis reduces the vitality of *H. contortus* L3 (12). Those reports coincide with our results which demonstrated that 53.6% of L3 withstand once drying for a period of 3 hours. Moreover, twice dehydration does not cause a significantly harm and many of larvae (72.2%) retain vital.

CONCLUSION

Freezing at -18ºC is the most important factor affecting the survival rate of *H. contortus* L3 than temperatures of -4ºC and 40ºC as well as desiccation. Therefore, the climate conditions in Bulgaria favor the prolonged survival period of larvae on pastures during all seasons. Moreover, the ability of L3 to perform horizontal and vertical migration further multiplies the risk of occurrence of haemonchosis among small and large ruminants during summer.

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