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EFFECT OF TEMPERATURE AND DESICCATION ON SOME EXOGENOUS STAGES OF *HAEMONCHUS CONTORTUS*

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Summary

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Haemonchus contortus (*Nematoda*: *Trichostrongylidae*) constitutes a major part of gastro-intestinal helminthic fauna of ruminants and is one of the most pathogenic member of this family causing serious economic losses at global scale. Part of its life cycle takes place in the environment under different climatic conditions. Resistance rate of pre-infective stages of *H. contortus* to temperature and humidity variations could be used to predict its occurrence among ruminants. The aim of present study was to investigate the effect of temperature and desiccation on eggs of *H. contortus* under laboratory conditions. A pure infection by *H. contortus* was reproduced in experimental lambs and fresh eggs were obtained from feces by routine sugar flotation technique. Eggs were placed at various temperatures (–4 ºC; −18 ºC; 40 ºC) and desiccation. The results clearly showed that embryonated eggs were more resistant to both high and low temperatures than unembryonated eggs. Desiccation influences destructive on exogenous development stages.

Key words: eggs, desiccation, *Haemonchus contortus*,temperature

INTRODUCTION

The temperature and humidity are the most important factors exerting a marked effect on various stages of exogenous development of gastrointestinal strongylids. According to Silverman & Campbell (1959), *H. contortus* eggs which contain first stage larvae are more cold resistant than those in early stages of development. Todd *et al*. (1976) tested the effect of 4 ºC on non-parasitic stages of *H. contortus*. The authors found that less than 1% of unembryonated eggs followed by first and second larvae and embryonated eggs remained vital on days 8, 64, 32 and 128, respectively. The authors revealed that low temperatures affected most adversely the eggs having undergone embryonic development. According to Mizelle & Berberian (1953) the embryonation of *H. contortus* eggs was completely postponed at 4 ºC. At the same temperature Tripathi (1980) also found no egg development during a thirty-day observation period. Moreover, *H. contortus* eggs placed under the same conditions for 3 days delayed their development and less than 60% remained viable. No vital eggs have been obtained during a new 12-day exposure period under the same conditions (McKenna, 1998). In a similar study, Jasmer *et al*. (1986) demonstrated that only 1.3% of unembryonated *H. contortus* eggs developed to the subsequent stages at −18 ºC for 15 h followed by incubation at 30 ºC. The second part of experiment revealed egg hatchability rate of 4% under the same conditions. High temperatures also were harmful for *H. contortus* egg development. According to Khatun *et al*. (2013) egg development and hatching have been partially decreased at a temperature over 35 ºC.

MATERIAL AND METHODS

Eggs were acquired from lambs experimentally infected with *H. contortus*. Prior to the study, an approval for using animals in experiment was obtained from the Bulgarian Food Safety Agency (permit No. 107/10.07.2014). Fresh and embryonated eggs were exposed to different temperatures and desiccation (Table 1). The key criteria in assessing the harmful effects were changes in structure of eggs such as destruction of blastomeres and first stage larva within egg shell and changes of egg development.

RESULTS

Freezing of unembryonated *H. contortus* eggs at −4 ºC or −18 ºC for 24 h did not visibly altered their structure. Eggshells were not damaged and disturbances in the integrity of blastomeres were not been observed. Larvae forming within eggs incubated at 27 ºC were not detected on the 3rd day of temperature exposure. Similar results were also found in embryonated eggs. Larvae were not destroyed but hatching did not occur until the 3rd day of incubation at 27 ºC.

Table 1. Influence of temperature and humidity on *H. contortus* eggs

	Influence
1	Freezing at −4 ºC and −18 ºC for 24 h followed by cultivation at 27 ºC for 3 days
2	Heating at 40 ºC for 7 days
3	Drying at room temperature for 2 and 6 h followed by hydration and cultivation at 27 ºC for 3 days

Heating the unembryonated eggs at 40 ºC for 7 days resulted in development in 8.4% on day 3 of cultivation. Hatchability of embryonated eggs was 60% and 69% on days 2 and 3, respectively (Fig. 1). No larval hatching was detected between the 4th and 7th day after incubation.

Drying of unembryonated eggs at room temperature for 2 to 6 hours caused obvious destruction. Larval formation did not occur by the 3th day after placing the eggs under favourable humidity and temperature. A similar result was observed in embryonated eggs placed under the same conditions. After moistening, the first stage larvae were immobile and hatching did not occur within 3 days of cultivation.

DISSCUSION

Our results showed the freezing significantly influenced eggs development. It is in agreement with data of Todd *et al.* (1976) who affirmed that the low temperature had a strongest negatrive effect on unembryonated *H. contortus* eggs fol-

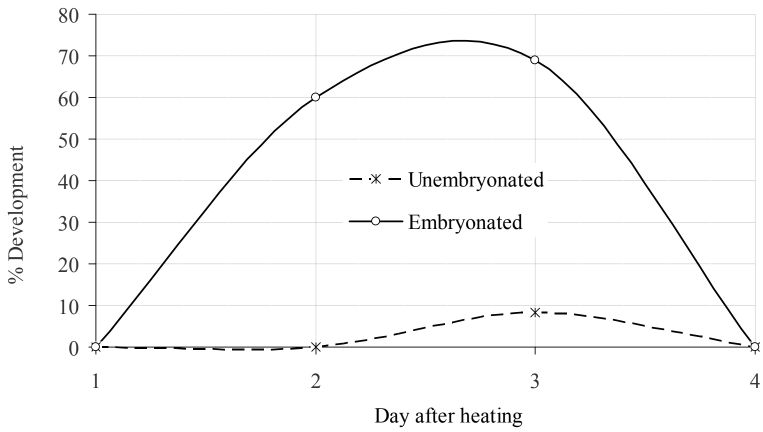

Fig. 1. Development of *H. contortus* eggs at 40ºC for 4 days.

lowed by first and second stage larvae and embryonated eggs. Authors found out under 1% development and vitality of fresh or embryonated *H. contortus* eggs at 4 ºC on day 8 and 128, respectively. We also agree with the opinion of McKenna (1998) who confirmed an irreversible inhibition of embryogenesis of *H. contortus* eggs placed at 4 ºC for 15 days. In our opinion, the probable reason was a change in the microstructure of egg blastomeres. The results of this study are different from those published earlier. Data on similar survey showed a high tolerance of *H. contortus* eggs to freezing (Silverman & Campbell, 1859). Authors reported the embryonated eggs resisted temperatures between –1 ºC and –2 ºC for 2 months.

Our results also demonstrated the high temperature greatly reduced the unembryonated egg development but at a lesser extent, larval hatchability. Similar experiments mentioned that the temperatures above 40 ºC destroyed not only eggs but also first stage larvae of most gastrointestinal strongylids (Crofton, 1965; Tripathi, 1977; Islam *et al.*, 2005). It is probably because heating causes changes in the structural order of protein molecules – the main reason of egg and larval mortality at high temperatures.

Our results indicated that the desiccation was the most important factor affecting the development of *H. contortus* eggs. This opinion is in line with data presented by Wharton (1982) who studied the influence of humidity on egg development in feeces or coproculture and reported that desiccation led to fast destruction of unembrionated strongylid eggs. These results should be related to some general ecological specifics of external development of gastrointestinal stongylids. It is well known that the faeces contain moisture and parasite eggs are adapted to it during the evolution. Several hours after defecation, the surface of faeces dries thereby creates a barrier preventing further fluid losses. Non-parasitic stages of strongylid development use this „shelter", which provides an optimum moisture to develop the infectious larval stages, which are considerably more stable. Only when resistant infective stages were fully formed and in presence of adequate external moisture, the larvae leave faeces and fall to the grass of the pasture. This statement explains the low stability of *H. contortus* eggs, especially unembryonated ones, which are highly dependent on the amount of moisture. Furthermore, blastomere division and larvae forming are considered to be the most critical moments of exogenous development of gastro-intestinal strongylids.

CONCLUSION

Humidity is a more important factor for development of both fresh and embryonated *H. contortus* eggs than high temperature. Freezing damages significantly not only blastomeres but also first larvae within egg shell. Based on this, it may be assumed that the warm and dry summer and cold winter are able to delay or interrupt the life cycle of *H. contortus* and the other gastrointestinal strongylids.

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