Journal of Animal and Veterinary Advances 6 (3): 458-460, 2007

Experimental Study on the Causative Agents of Hypobios is of *Haemonchus contortus* in Sheep in Iran

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Abstract: For determination the inducing factors of arrested development of 3th stage larvae of *Haemonchus contortus* as early L4 in sheep in 2 out of 4 climatic conditions of Iran, 24, six month old native sheep (shall breed) were randomly allocated into 3 equal groups I, II and III.Each sheep in each group received 10000 local isolate of *H.contortus* larvae not known to exhibit hypobiosis as follow: (i)Freshly collected larvae were given to group I, (control group).(ii)Larvae stored at 8-10 ^{oc} and 70% humidity for six weeks (simulating autumn climatic conditions of zone I) to group II.(iii)-larvae exposed to 30-350^{oc} and 40% humidity for six weeks (simulating autumn climatic conditions of zone III) to group III.Two Iambs in each group at 12, 21, 35 and 60 days post infection were necropsies and the number of adult and larvae were counted. Our findings suggest that the rate of arrested larvae in group II were statistically significant ($p<0.01$), whereas in two other groups only a very small number of larvae were subjected to hypobiosis. Meanwhile the number of adult worms recovered from group I receiving fresh larvae was higher than the other two groups.

Key words: Hypobiosis, *Haemonchus contortus*, sheep, Iran, larvae

INTRODUCTION

The capacity to enter a state of hypobios is has evolved in *Haemonchus* contortus to a higher degree than any other trichostrongylid parasites (Dunn, 1978). Contradictory results are reported on the mechanisms of hypobios is in different regions of the world. No hypobiosis was detected in Brazile (Charles, 1989) and Egypt (Azazy, 1990) whereas in temperate regions such as England (Connan, 1971) Newzaland (Burnsdon, 1973) Canada (Blitz and Gibbs, 1972; Gibbs, 1973) and the U.S.A. (Herd *et al*., 1984) entire *H.contortus* population have been observed in hypobiosis in naturally infected lambs. Meanwhile it was shown that hypobiosis is independent on immunity (Anderson *et al*., 1965) dependent on immunity (Balic *et al*., 2000) depending on decline in temperature in autumn and winter (Michel *et al*., 1973) photoperiod (Cremer *et al*., 1975; Gibbs, 1973) and nematode strain (Eysker, 1997) Although the role of photoperiod remain equivocal (Gibbs, 1973). In Connan experiments (1975) neither cold (4^{oc}) nor autumn conditions of declining temperature and photoperiod were key factors in the induction of hypobiosis (Cannon, 1975) Haemonchosis is prevalent among wild and domestic ruminants of Iran (Eslami and Fakhrzadegan, 1997; Eslami and Fazy, 1975; Eslami and Nabavi, 1976; Eslami *et al*., 1980) and is reported from man as well (Ghadirian and Arfaa, 1973). Natural hypobiosis of some trichostronglids of sheep rather than*H.contorus* was occurred in southern part of Iran (Zone II) (Michel and Hooshmand, 1978).

In the present study the effect of artificial conditioning on infective larvae of *H.contortus* was examined in an experiment involving 24 native lambs. The study was carried out by a local isolate of *H.contortus* not known exhibited hypobiosis.

MATERIALS AND METHODS

Donor sheep: Two native nematode free one year old female sheep were infected with 10000 third stage larvae of *H.contortus* and were kept in Animal Husbandry Centre, Tehran Veterinary Faculty and were fed manually during the experiment. Three weeks post infection fecal egg count was performed using flotation method.

Fecal culture: Enough daily fecal samples were directly collected from the rectum.They were then broken down and mixed with vermiculite and were incubated at dark at 22^{oc} and 70-80 % humidity for10 days. Baermann funnel was used to remove the larvae from the cultured feces.The mean number of larvae was calculated according to 10 aliquots (0.05 mL each) and their identification was performed using the key presented by Thomas and Probert (1993).

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Weather data: Based on the mean of monthly rainfall, humidity and temperature, Iran is divided into 4 climatic zones of which simulating autumn climatic conditions of two zones:

- Zone I-Caspian Sea zone including the costal plains and northern aspect of the Alborz range facing the Caspian Sea. (Mean annual rainfall 40-150 cm, mean annual temperature 8-26^{oc} and high humidity in most months of the year).
- Zone III-Persian Gulf lowland extending northward along the basin of the Tigris river bordering Iraq. (Mean annual rainfall 20-30 cm, mean annual temperature 13-36^{oc} and high humidity in spring and summer).

Experimental design: Twenty four lambs free of gastrointestinal nematodes were randomly allocated into three groups of 8 lambs and each received 10000 larvae exposed to following conditions:

- Group I-Freshly collected larvae (control group).
- Group II-larvae exposed to 10^{oc} and 70% humidity for six weeks (simulating t autumn conditions of zone I).
- Group III-larvae stored at 30-35^{oc} and 40% humidity for 6 weeks (simulating autumn conditions of zone III).

Two lambs from each group were necropsied at 12, 21, 35 and 60 days postinfection (p.i). The abomasums were opened along the greater curvature and were washed to remove safely the adult and immature form of *H. contortus*. To count the number of worm and larvae the whole contents were examined little by little under the dissecting microscope.

Statistics: Obtained data were entered in SPSS and SAS 12/6 package and were analyzed by one way analysis of variance and Duncan method.

RESULTS AND DISCUSSION

The results of our study are summarized in Table 1. No adult worm was found in sheep necropsied 12 days P .i. and all collected larvae at this stage were taken as developed but not hypobiotic larvae. The rate of larval mortality in all group was high and was 72.51, 84 and 97% in groups I, II and III, respectively. Although hypo bios is of third stage larvae of *H. contortus* was observed in all groups but in group I and III a very small proportion of the total *Haemonchus* larvae were subjected to hypobiosis, whereas in group II the number of arrested development of larvae was highly significant ($p<0.01$). Accordingly it higher freshly cultured larvae developed to adult (49%) in group I in comparison with those of two other groups.

Table 1: Total worms, Percent recovery and percent hypobiosis of *H. contortus* in 24 necropsied Iambs at days 12, 21, 35 and 60 days P.i.

Lambs groups	Total worms	Recovery%	Hypobiosis%
I	2160	21.6	0.0/1
II	1280	12.8	0.0/8
III	197	1.97	0.0/98

Present study indicates that simulation of autumn conditions of zone II w ould be the factors suitable for third-stage larvae of *H. contortus* subjected them significantly to a level of hypo bios is in the host. Therefore, it can postulated that under similar weather conditions to group II of this experiment, natural hypobiosis of *H. contortus* in sheep may occurs in that region (zone I). Even in the latter region, number of *H. contortus* larvae underwent hypobiosis in comparision with Gatongi *et al.* (1996) and Fernandez *et al.* (1999) were very low. (between 19-54% and 31.1-71.2% respectively). Generally speaking our results are in line with those of Eysker (1981) in the Netherland and Mckenna (1973) in Newzland who showed hypobiosis with L_3 of *H. contortus* stored at 15-16^{oc} for 30-38 days and at 5^{oc} and 21^{oc} for 80 days respectively. In the case of Iambs received freshly cultured larvae our results are in contrast to the findings of Ogunsusi (1979) in Nigeria who reported over 90% hypobiosis in lambs infected with larvae fteshly cultured at 25-30^{oc} for 7-12 days.

There is no report on the involvement of both temperature and humidity on the induction of hypobiosis. Thus, it is not possible to determine the accurate role of each of these factors on the induction of hypobiosis in the present study. High temperature (30-35^{oc}) and low humidity (40%) although are autumn conditions in zone III of Iran but could not induce hypobiosis in *H. contortus* larvae. It can postulate that high temperature suppress the viability of the larvae to a degree that they are not able to undergo hypobiosis, even development to adult, because lowest number of adult worm and very low number of hypobiotic larvae were obtained in this group.

In addition to simulating autumn climatic conditions, it seems some other factors could act as the causative agents of hypobios are in some parts of Iran. In Fars province, southern part of Iran (zoneII) natural hypobiosis of *Trichostrorgylus colubriformis. marshallagia marshalli Nematodirus filicollis* and *Dictyocaulus filaria* of sheep has been reported. These larvae were subjected to hypobiosis before migration of sheep to summer pastures in March and developed to adult after returning to winter pastures in September (Michel and Hooshmand, 1978). Therefore it seem, in a vast country like Iran with different climatic conditions, different factors govern hypobiosis. Still the causative factors of hypobios is of nematode are not well identified, but environmental conditions such as temperature, humidity, photoperiod, nematode strain and immunity

could act as factors responsible for hypobiosis. Although Capitini *et al.* (1990) believes that hypobiosis in *H.contortus* is an obligatory mechanism to live and no external stimuli is needed to reach this point.

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