Global Veterinaria 16 (3): 255-260, 2016
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2016.16.03.10311

Effect of Steppic Environment on Hypobiose of Abomasal Trichostrongylid Nematodes in Lambs of Batna (North-East Algeria)

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Abstract: Hypobiosis in abomasal trichostrongylid nematodes was studied in thirty permanent lambs of a large farm in the North-East of Algeria. The climate is steppic characterized with cold winter and hot and dry summer. From February 2008 to February 2009, the sentinel lambs were monthly monitored for trichostrongylids using nematode faecal egg counts. Two of the original thirty lambs were necropsied every two month after being held in pens for three week. A higher percentage of fourth stage larvae (L4) reaching 48% of the total worm burden was recorded in abomasal contents in June. The *Teladorsagia* constituted the highest percentage of L4 (71%), followed by *Trichostrongylus* (17.4%) and *Haemonchus* (11.6%). The significant higher level of excretion of trichostrongylid eggs and the higher number of adults worms in August dominated by *Teladorsagia circumcincta*, was due to the release of L4 inhibition.

Key words: Hypobiosis · Abomasum · *Teladorsagia* · Steppic · Climate · Lambs · Algeria

INTRODUCTION

The life cycle of many endoparasites can be delayed by the development of free-living larvae stages or a developmental arrestment in the hoste referred to as hypobiosis. It enables the parasite to have available large number of infective forms at moments in the host life cycle that coincide with the presence of susceptible young lambs, thus ensuring transmission form one generation to the next [1]. Hypobiosis of a short or intermediate duration increases the destabilizing effect on populations, whereas hypobiosis of a duration of five months or more can stabilize interactions, irrespective of the regulation of the host population dynamics [2]. Hypobiosis in trichostrongylid nematodes is mostly recorded in regions with long cold winters [3-7]. The cold winter hypobiosis is characterized by larvae in the mucosae and the duration is long, more than four months. Under tropical climates with a dry season, hypobiosis has been recorded during unfavourable season [8,11]. In dry season hypobiosis, the duration was short (A month or two) and the larvae were in the abomasum content for most of them. In mountain regions of Morocco, winter and summer hypobiosis were recorded [8]. The aim of this paper was to assess the prevalence of hypobiosis along the year in a steppic environment. Due to the cold winters and dry and hot summers, it may be expected that both hypobiosis (Winter and summer ones) are present under this type of climate.

MATERIALS AND METHODS

Farm and Climate of Study Region: The studied farm was located in the Batna region of North-East Algeria. The farm managed 1200 Ouled Djallel sheep, which were partially maintained in a sheepfold in winter and grazed on communal pastures with animals owned by neighbouring small-holder farms from October to May and then on cereal stubble and crop residues from June to September. Their grass diet was partly supplemented from October to February and the main lambing period was in autumn. Climatic data are presented in Figure 1. According to Emberger [12] Batna is located in a semi-arid bioclimatic level with cold winters and could be characterized as steppic climate according to the criterion of Viers and Vigneau [13] the steppe yearly rainfalls (R) are below

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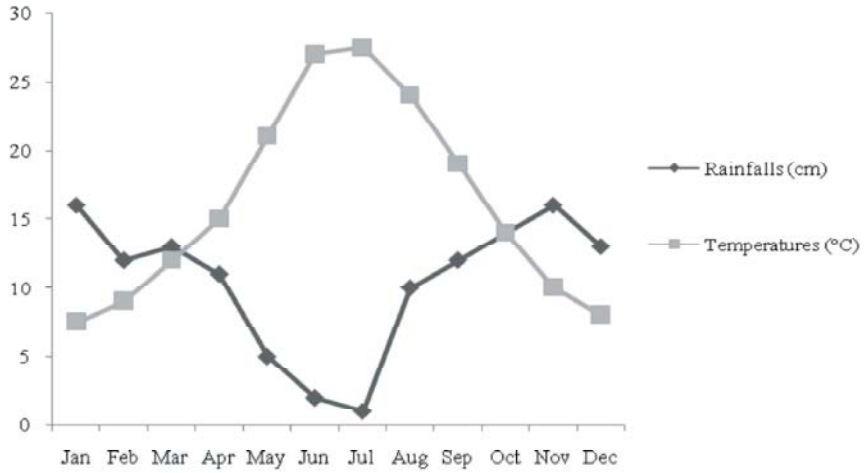

Fig 1: Monthly average temperatures and rainfalls of study region (Batna): Meteorological data of 34 years provided by the meteorological station of Batna.

40cm/year and related to yearly average temperatures (T in °C) in the following ways: $R_{in\ cm} \square 2$ t. the coldest month in the area was January (Average 5,3°C) and the hottest was July (Average 25°C). The drought period extended from June to September.

Parasitological Analyses: Thirty weaned lambs were selected among available lambs and were used as sentinels to monitor the gastro-intestinal nematodes (GIN) infection between February 2008 and February 2009. No anthelmintic treatments were given to the experimental animals during the study period. Faecal eggs counts (Number of eggs per gram of faeces: EPG) were carried using a modified McMaster method with a saline solution (1.18 specific gravity) sensitive to 15 EPG. In the event that McMaster method did not detect any egg, a flotation method was also used determined to be sensitive to 7.5 EPG. Two lambs were necropsied every two months to identify the species of GIN and their larvae (L4 larvae and juvenile fifth stage). The necropsies were conducted according to MAFF guidelines (1986) and the GIN species were identified based on criteria described by Skrjabin *et al*. [14]. The contents of the abomasum were sedimented in water several times until the supernatent was free of debris and the adults and inhibited larvae of the GIN could then be identified and counted on a 1/5 to $1/10^{th}$ aliquot. The abomasal mucosa was digested by incubation at 37°C for 20 hours in pepsin acid solution (10 gram pepsin, 30 ml hypochloric acid and 1000 ml distilled water as described in Giangaspero *et al*. [9]). The resulting suspension was then sieved through a 32µm mesh and the larvae were counted.

Statistical Analyses: The dynamics of the infection intensity (Based on EPG or worm counts) were analysed using non-parametric Kruskall and Wallis tests. Additionally, a logarithmic transformation for an analysis of variance (ANOVA) was carried out and followed by a *post hoc* Newman and Keuls test to classify the months into high or low infection based on EPG in faeces, adult or larvae at necropsy. All the statistical analyses were performed using SPSS 11.5 software. The percentages of L4 and juveniles are calculated by the following formulae:

Percentage of specie in L4 = number of L4 specie X 100/ number total of L4 ;

Percentage of L4 in community = number L4 X 100/ number (L4 + juveniles + adults)

Percentage of specie in juveniles = number of juvenile species X 100/ number total of juveniles

Percentage of juvenile in community = number juvenile X 100/ number (L4 + juveniles + adults).

RESULTS

Dynamic of Eggs Faecal Excretion: The dynamics of faecal excretion of eggs for *Marshallagia marshalli* and other digestive strongyle (ODS) was showed in Figure 2. According to the Kruskall and Wallis test, excretion varied significantly between the months ($p = 0.000$). The univariate ANOVA indicated that excretion of ODS was significantly higher in August compared to all other

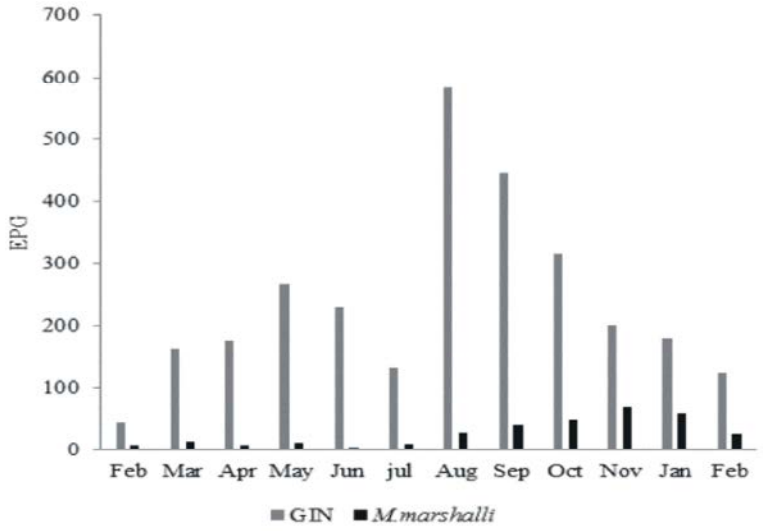

Fig. 2: The egg excretion of gastro-intestinal strongyles (GIS) and *Marshallagia marshalli* in lambs during one year.

Table 1: Composition of the abomasal worm community (larvae L4, juveniles and adult worm) of lambs during the six occasions of necropsy

Month	April	June	August	October	December	February
Number of adult worms						
Teladorsagia circumcincta	107	670	2366	215	37	12
Teladorsagia trifurcata	30	0	91	60	22	16
Haemonchus contortus	0	0	917	271	78	60
Marshallagia marshalli	87	230	1099	1561	689	188
Trichostrongylus vitrinus	0	0	140	69	0	8
Trichostrongylus axei	0	0	0	69	6	0
Total	224	900	4613	2245	832	284
Total L4	58	860	2,6	0	2,3	0
Percentage of species in L4						
T. circumcincta and other Ostertagiinae	53,4	71	14,8	0	50	0
Haemonchus contortus	0	11,6	11,1	0	0	0
Trichostrongylus sp.	46,6	17,4	74	0	50	0
Percentage of L4 in community (L4 / (L4+Juveniles+Adults))	20,5	48,3	2,6	0	2,3	0
Total juveniles	0	20	350	0	10	0
Percentage of species in juveniles						
T. circumcincta and other Ostertagiinae	0	100	100	0	0	0
Trichostrongylus sp.	0	0	0	0	100	0
Percentage of Juveniles in community (Juvenile/ (Juveniles+L4 +Adults))	0	1,1	6,9	0	1	0

months (p=0.001). Excretion of *Marshallagia marshalli* was significantly higher in autumn, particularly in November (p=0.02).

Seasonal Dynamics of GIN Species: The different species of adult worms and their larvae observed in the six occasions of necropsy are reported in Table 1. Statistical analyses with ANOVA showed a significant differences of the numbers of Ostertagiinae and *Haemonchus contortus* fourth stage larvae (L4) between months (p=0.008 ; p=0.001 respectively). The numbers of L4 of Ostertagiinae and *H. contortus* were found to be highest in June (ANOVA and *post hoc* Newman-Keuls test). The most abundant species of L4 observed was the Ostertagiinae in June accounting for 71% of the L4 burden and 48% total worm burden (L4/ (L4+ juveniles+adults)) (Table 1). The percentage of *H. contortus* L4 in June was 11.6%. In August, the percentage of L4 in community decreased accounting for only 2.6% of the total worm burden and the number of juveniles increased and reached a peak of 350 worms representing 6.9% of the worm burden. A significant peak of juvenile Ostertagiinae was recorded in August (ANOVA (p=0.01). *Trichostrongylus sp.* appeared to be stable at low levels throughout the year.

There were two dominant adult abomasal nematode species throughout the summer period (In August) including *T. circumcincta* (p=0.07) and *H. contortus* (p=0.06) (Non-parametric Kruskall and Wallis test). *Marshallagia marshalli* was the predominant species detected in autumn-winter.

Seasonal Dynamics of Worm-Burdens: No L4 were found in lambs examined in winter (0% in December and February 2009). In spring, these larvae appeared in April and the percentage (L4/ (L4+ juveniles +adults)) was 20.5% and 48.3% in the beginning of summer (June) (Table 1). At this point, the L4 were found only in the contents of abomasum. In the middle of summer (August), the percentage of L4 in the worm burden decreased to a negligible 2.6%. However the percentage of juveniles increased from 1,1% in June, to 6,9 % in August and consequently a peak of 4613 adult GIN was reached in August.

DISCUSSION

The main abomasal worm species found in the area were *T.circumcincta* and *M.marshalli*. The same fauna have also been recorded on farms in eastern Algeria [15], Iraq [16], Syria [9], Morroco [8], differing sites studied in a meta-analysis of the steppic region [17] or other similar regions [18]. Under the same steppic climate, the faecal egg excretion dynamics realized in Algeria by Bentounsi *et al*. [15] and Boulkaboul and Moulaye [19] showed two peaks of excretion of eggs, one in Spring and one in Autumn. This differed slightly from the dynamics of EPG observed in this study as the single highest peak occurred from August to September. It is possible these discrepancies result from the study of lambs exposed to differing anthelmintic treatments regimes; where they were untreated in the present study, this was not the case in the other studies. The anthelmintic treatments in these areas were carried out in July or August when the lambs were at the peak of nematode egg excretion and then in winter; all sheep were treated. The dynamics of the L4 (mostly *T.circumcincta*) was also seasonal, reaching a peak in June to represent 48% of the total worm burden. At this moment, the L4 were found in the abomasal contents and not in the mucosa, which was the usual situation of winter-arrested larvae. Large numbers of summer-arrested larvae had also been found in Syria [9] where they represented 85% of the total worm burden and other regions with a long dry summer i.e. Saudi Arabia [20], Eastern Ethiopia [21]. In the Middle-Atlas of Morocco, both winter and summer peaks of L4 were recorded over three consecutive years [8]. The L4 summer peaks in the present study did not correspond exactly to the classification of summer-hypobiosis in Trichostrongylid nematodes given the L4 here were not found in the mucosae [6]. However according to Michel [22] defining whether hypobiosis had occurred could be evidenced by one of two factors: a) the presence of the same immature stage when there had been no sudden uptake of infective larvae, b) the continued presence of immature stages after with drawal from infection when sheep were kept in sheep pen for a length of time longer than the pre-patent period. The results from this study clearly meet the first condition, yet it was more difficult to verify the second condition given we had no knowledge of the availability of infective larvae on pasture. Nonetheless, the dynamics of infection observed here (Highest Faecal egg count in August) and the stage composition of worm burden (Highest percentage of L4 in June) provided strong evidence that arrested development had occurred. This arrest appeared in June when the temperature was at its highest and the rainfall levels were negligible. Both high temperatures and low moisture present extremely difficult conditions for the development of consequent free-living stages in GIN [23]. It appeared larval arrest was largely triggered by climatic variables in both winter [24] and summer hypobiosis. In this study, 71% of arrested L4 were represented by Ostertagiinae in June. It was likely these largely corresponded to the species *T. circumcincta*. The *Marshallagia* adult population was low in summer and no hypobiosis was detected. Low levels of *H.contortus* L4 were also observed in June (11.6%) but was unlikely this corresponded to hypobiosis, but rather to the normal turnover of infection. Interestingly, *H. contortus* was the most commonly recorded GIN species to undergo hypobiosis in summer of dry/hot areas [10, 11, 20, 25, 28]. It was possible the differing results in the present study might be attributed to the cold winters which interrupted the hot summers in the study area and might explain the low percentage of *H. contortus* and the absence of summer hypobiosis for this species.

ACKNOWLEDGEMENTS

This work was in part funded by an National Research Programme (PNR) from the Ministry of Higher Education and Scientific Research (PNR, I/U250, 233). We thank the heads of the El Mader farm of Batna,

mainly BOUBIDI Houcine, TARFAYA Nourredine without forgetting the health animal technician KISRI Slimane for their support for the realization of this work. Caroline CHYLINSKI commented an earlier draft of the paper and revised the English language.

REFERENCES

1. Gibbs, H.C., 1982. Mechanisms of survival of nematode parasites with emphasis on hypobiosis. Veterinary Parasitology, 11: 25-48.
2. Gaba, S. and S. Goubiere, 2008. To delay once or twice: the effect of hypobiosis and free-living stages on the stability of host-parasite interactions. Journal Royal Society, 5: 919-928.
3. Blitz, N.M. and H.C. Gibbs, 1972. Studies on the arrested development of Haemonchus contortus in sheep: I. The introduction of arrested development. International Journal of Parasitology, 2: 5-12.
4. Capitini, L.A., K.E. McClure and R.P. Herd, 1990. Effect of environmental stimuli on pre-infective and infective stages of Haemonchus contortus in the Northern United States for the induction of hypobiosis. Veterinary Parasitology, 35: 281-293.
5. Langrova, I., K. Makovcová, J. Vadlejch, I. Jankovská, M. Petrtıl, J. Fechner, P. Keil, A. Lytvynets and M. Borkovcová, 2008. Arrested development of sheep strongyles: onset and resumption under field conditions of Central Europe. Parasitology Research, 103: 387-392.
6. Michel, J.F., M.B. Lancaster and C. Hong, 1974. Studies on arrested development of Ostertagia ostertagi and Cooperia oncophora. Journal Comptes Pathology, 84: 539-554.
7. Sargison, N.D., D.J. Wilson, D.J. Bartley, C.D. Penny and F. Jackson, 2007. Haemonchosis and teladorsagiosis in a Scottish sheep flock putatively associated with the overwintering of hypobiotic fourth stage larvae. Veterinary Parasitology, 147: 326-331.
8. Cabaret, J., 1984. Seasonal changes in the abomasal nematodes of naturally infected ewes in Moulay Bouazza (Morroco). Veterinary Parasitology, 15: 47-56.
9. Giangaspero, M., E.F. Thomson, G. Orita, F.A. Bahhady and C. Rhodes, 1992. Effect of gastrointestinal parasite and lungworm control on survivability and fertility of ewes in farm flocks in Syria. Tropical Animal Health Production, 78: 594-597.
10. Ogunsusi, R.A. and M. Eysker, 1979. Inhibited development of trichostrongylids of sheep in Northern Nigeria. Research in Veterinary Science, 26: 108-110.
11. Vercruysse, J., 1985. The seasonal prevalence of inhibited development of Haemonchus contortus in sheep in Senegal. Veterinary Parasitology, 17: 159-163.
12. Emberger, L., 1930. Sur une formule applicable en géographie botanique. Comptes Rendus Académie Sciences, 191: 389-390.
13. Viers, G. and J.P. Vigneau, 1990. Eléments de climatologie. Nathan Ed.
14. Skrjabin, K.I., N.P. Shikobalova and R.S. Shultz, 1954. Trichostrongylids of Animal and Man. Nat Sci Foun, Department of Agriculture, Washington, pp: 483.
15. Bentounsi, B., A. Mecif and K. Kohil, 2001. Evolution du parasitisme ovin sur un élevage de la région du Khroub. Approche par les méthodes coproscopiques. Sciences et Technologies, 16: 51-54.
16. Altaif, K.I. and W.H. Issa, 1982. Seasonal fluctuations and hypobiosis of gastrointestinal nematodes of Awassi lambs in Iraq. Parasitology, 86: 301-310.
17. Meradi, S., B. Bentounsi, I. Zouyed and J. Cabaret, 2011. The steppe species of gastrointestinal nematodes of small ruminants, with a focus on Marshallagia: climate as a key determinant. Parasite, 18: 261-269.
18. Suarez, V.H. and J. Cabaret, 1991. Similarities between species of the Ostertaginae (Nematoda: Trichostrongylidae) in relation to host-specificity and climatic environment. Systematic Parasitology, 20: 179-185.
19. Boulkaboul, A. and K. Moulaye, 2006. Parasitisme interne du mouton de race Ouled Djellal en zone semi-aride d'Algérie. Revue Élevage Médecine Vétérinaire Pays Trop, 59(14): 23-29.
20. El-Azazy, O.M.E., 1995. Seasonnal changes and inhibited development of the abomasal nematodes of sheep and goats in Saudi Arabia. Veterinary Parasitology, 58: 91-98.
21. Menkir, M.S., U. Arvid and J. Wallerp, 1974. Epidemiology and seasonal dynamics of gastrointestinal nematode infections of sheep in a semi-arid region of eastern Ethiopia. Veterinary Parasitology, 143: 311-321.
22. Michel, J.F., 1974. Arrested development of nematodes. In: Advances in Parasitology; 12: 279-365. Ed B. Dawes Acad Press London.

23. O'Connor, L.J., S.W. Walkden-Brow and L.P. Kahn, 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Veterinary Parasitology, 142: 1-15.
24. Fernández, A.S., C.A. Fiel and P.E. Steffan, 1999. Study on the inductive factors of hypobiosis of Ostertagia ostertagi in cattle. Veterinary Parasitology, 81: 295-307.
25. Allonby, E.W. and G.M. Urquhart, 1975. The epidemiology and pathologic significance of Haemonchosis in a merino flock in east Africa. Veterinary Parasitology, 1: 129-143.
26. Chiejina, S.N., B.B. Fakae and B.O. Eze, 1988. Arrested development of gastrointestinal trichostrongylids in goats in Nigeria. Veterinary Parasitology, 28: 103-113.
27. Gatongi, P.M., R.K. Prichard, S. Ranjan, J.M. Gathuma, W.K. Munyua, H. Cheruiyot and M.E. Scott, 1998. Hypobiosis of *Haemonchus contortus* in natural infections of sheep and goats in a semi-arid area of Kenya. Veterinary Parasitology, 77(1): 49-61.
28. Pandey, V.S., M. Ndao and V. Kumar, 1994. Seasonal prevalence of gastrointestinal nematodes in communal land goats from the highveld of Zimbabwe. Veterinary Parasitology, 51: 241-248.