



Full Length Article

Contamination of Infective Larvae of Gastrointestinal Nematodes of Sheep on Communal Pasture

I.R.M. AL-SHAIBANI¹, M.S. PHULAN, A. ARIJO AND T.A. QURESHI[†]

Department of Veterinary Parasitology, Sindh Agriculture University, Tando Jam, Pakistan

[†]Department of Veterinary Pharmacology, Sindh Agriculture University, Tando Jam, Pakistan

¹Corresponding author's e-mail: dr_ibra67@yahoo.com

ABSTRACT

The prevalence of the infective larvae of gastrointestinal nematodes on pastures naturally contaminated with faeces of permanently grazing sheep in Hyderabad areas was monitored during 2004-2005. The herbage samples were collected from selected sites from the Hyderabad district viz., Hyderabad city, Tandojam, Tando Allahyar, Tando Muhammad Khan and Halla at the fortnightly intervals for 12 months and the infective larvae were identified and counted. The results revealed that the highest pasture larval counts were recorded in August, which coinciding with summer rainy season, whereas the lowest in January, which coinciding with dry winter season. Statistical analyses showed a significant ($P < 0.01$) influence of seasons on pasture larval counts. The infective larvae collected from herbage samples were *Haemonchus contortus* Rudolphi, *Ostertagia circumcincta* Stafrimann, *Trichostrongylus* spp. *Cabbold Strongyloides papillosus* Chitwood, *Oesphagostomum columbianum* Molin and *Chabertia ovina* Molin. The infective larvae of *H. contortus* were the most prevalent. These findings may provide a tool for predicting pasture larval infectivity, which can be useful in designing control program for gastrointestinal nematodes in Hyderabad and other irrigated agro-ecological zones in the province of Sindh, Pakistan.

Key Words: Gastrointestinal nematodes; Availability; Pasture larval counts; Sheep

INTRODUCTION

Nematode infection is one of the major causes of economic losses in sheep production in the world (Suarez & Buseti, 1995). Small ruminants play an important role in the national economy of Pakistan. There is no organized sheep farming in the country except at some government livestock farms. The major share of national sheep population comes from the small flocks maintained by the landless families or small land holders in the villages (Lateef, 2002). Communal pasture is defined as land officially property of government or landholders and used by farmers to rear their livestock. It contains natural vegetation dominated by grasses and shrubs (Rinehart, 2006). In Pakistan, There are no permanent pastures for livestock and sheep flocks graze on canal, banks, roadsides and crop residues in fields.

The animals usually remain victim of disease due to underfed and poor management practices. The bacterial and viral diseases comes as outbreak and result in huge mortality, if proper therapy is not instituted at proper time. Helminths, however, are the permanent parasites and their prevalence has been reported very high throughout Pakistan (Mohiuddin *et al.*, 1984; Pal & Qayyum, 1992; Iqbal *et al.*, 1993). Among helminthes, gastrointestinal nematodes carry high importance because of their insidious and severe

pathological effects (Iqbal *et al.*, 2005b). Present control strategies are complicated by the problem of anthelmintic resistance, particularly gastrointestinal nematodes of sheep and goats. However, methods of controlling gastrointestinal nematodes parasites that do not rely on frequent anthelmintic treatment are urgently required (Waruiru *et al.*, 1998). A better understanding of the characteristics of the bionomics of the free-living stages of these parasites might lead to the development of control strategies aiming at improved managerial practices or directly at the free-living stages on pasture (Waruiru *et al.*, 1998; Ng'ang'a *et al.*, 2004). The survival and the transmission of infective larvae (L₃) from faeces to herbage depend upon many conditions i.e., humidity, temperature, amount and type of herbage around faeces and breaking pellets by mechanical factors (Fernandez *et al.*, 2001). In many tropical and temperate regions, the availability and number of free-living stages of gastrointestinal nematode parasites on the pasture follow seasonal fluctuations (Gibson & Everett, 1976 & 77; Tembely *et al.*, 1997; Cheah & Rajamanickam, 1997; Jithendran & Bhat, 1999; Waruiru *et al.*, 2001; Fernandez *et al.*, 2001; Ng'ang'a *et al.*, 2004). Since weather conditions vary from region to region, studies on the bionomics of the free-living stages of nematode parasites are needed in the planning for locally applicable control strategies (Na'ang'a *et al.*, 2004). The objective of this study was to investigate

seasonal availability of infective larvae of gastrointestinal nematodes of sheep on communal pasture of Hyderabad areas, Pakiatan.

MATERIALS AND METHODS

The study was conducted from May 2004 to April 2005 in Hyderabad district. This area is situated in irrigated agro-ecological zone in the province of Sindh and which irrigated by a canal network of the Indus River. Climatically, the study area is subtropical humid and receives average annual rainfall of about 129 mm. The average maximum temperature reaches 40.8°C in May and minimum 7.9°C in January. The relative humidity is highest (73%) in the month of August and lowest (50%) in the month of April. One year cycle is divided into four seasons i.e., winter (December-February), spring (March-April), summer (May-September) and autumn (October-November). Summer includes monsoon season (July-August).

The herbage samples were collected from five communal grazing pastures of Hyderabad district, namely, Hyderabad city, Tandojam, Tando Allahyar, Tando Muhammad Khan and Halla. The samples were collected at fortnight intervals for a period of 12 months. The infective larvae of gastrointestinal nematodes were recovered and isolated from herbage according the techniques described by MAFF (1986) and Hansen and Perry (1994). The herbage samples were placed into plastic container filled with tap water, a few drops of non-ionic detergent were added and the mixture was agitated by hand and left to soak overnight. After removing and rinsing the herbage from the containers, the water was poured onto sieve, where infective larvae were retained. The infective larvae were then transferred into a Baermann's fennel at room temperature, from where they were recovered on the next day and kept at 4°C until counted and identified. The infective larvae collected from herbage samples were *Haemonchus contortus* (Rudolphi, 1803), *Ostertagia circumcincta* (Stafrimann, 1894), *Trichostrongylus* spp., (Cabbold, 1894), *Strongyloides papillosus* (Chitwood & McIntosh, 1934), *Oesphagostomum columbianum* (Molin, 1861) and *Chabertia ovina* (Molin, 1861). They were identified and counted according the techniques described by MAFF (1986), Urquhart *et al.* (1996), Fernandez *et al.* (2001) and Krecek and Maingi (2004). The washed herbage samples were air dried at room temperature and weighed. The number of larvae was expressed as L₃ per kilogram of herbage dry matter (L₃ kg⁻¹ DM).

During the counting and identifying the larvae, there were many of free living and parasitic larvae of nematodes. The free living and parasitic larvae were differentiated on basis of that free living larvae retained iodine and were orange color, while parasitic larvae remained colorless or un-stained for a considerable period. Furthermore, parasitic larvae have sheaths discernible at the tail end and the

internal organs not developed well compared to free living nematodes (MAFF, 1986; Hansen & Perry, 1994).

Climatic data, such as temperature, rainfall and relative humidity were recorded at Meteorological station located within Sindh Agriculture University, Tando Jam campus and the required climatic data i.e., minimum and maximum temperature, relative humidity and rainfall for the study period were obtained every month from this station.

The data obtained from the pasture (herbage) larval counts, were analyzed using SPSS 15.0 program. The data of pasture larval counts were first logarithm transformed in the form log₁₀ (x+1) to stabilize the variance before analysis.

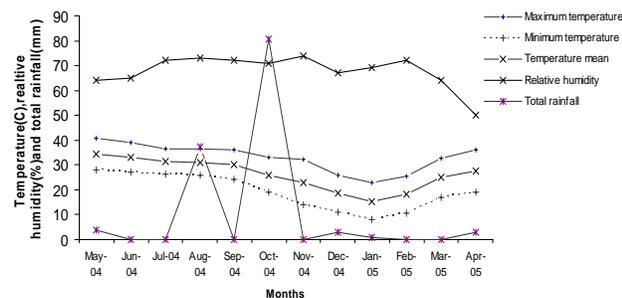
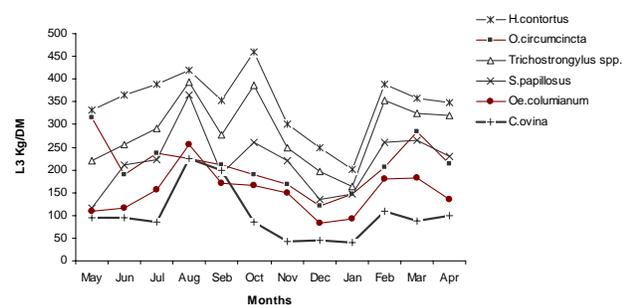
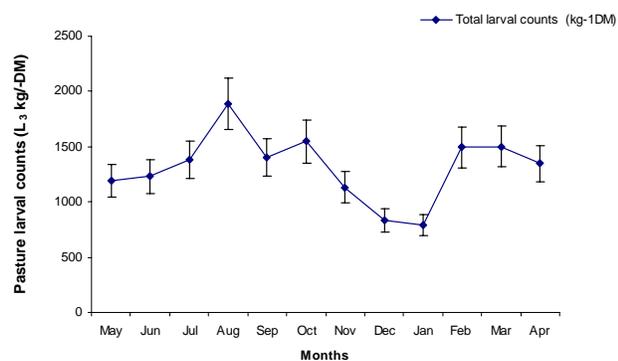
RESULTS

The meteorological data including monthly mean temperature, total rainfall and average relative humidity during the study period (2004-05) are showed in Fig. 1. The results of pasture larval counts and species of gastrointestinal nematodes recovered from herbage collected from selected sites of sampling areas are showed in (Fig. 2 & 3). The infective larvae species recovered from herbage were *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus* spp, *Strongyloides papillosus*, *Oesphagostomum columbianum* and *Chabertia ovina* in varying percentages.

Haemonchus contortus infective larvae were the most prevalent in study area. The prevalence was high from February to October and reached the peak of infectivity on August and October, then dropped sharply from November to lower level on January. The prevalence of *Trichostrongylus* spp. larvae followed the trend of *H. contortus* infective larvae. The numbers of larvae were high from February to October whereas it decreased from November to January. The prevalence of *O. circumcincta* larvae was high from February to September with a peak on May whereas it remained relatively low from October through January. The prevalence of *S. papillosus* larvae was high in two periods, in first period, from February to April and the second period from June to October with a peak infectivity on August, whereas it declined to minimum level in May and December. The infective larvae of *O. columbianum* and *C. ovina* were observed throughout of the year to varying degrees (Fig. 2 & 3).

The total larval counts and availability of infective larvae of gastrointestinal nematodes were the highest in August, which coinciding with summer rainy season, while declined to lowest level in January, which coinciding with dry winter season. During the other months of the year the pasture larval counts remained fluctuated as shown in Fig. 3.

Statistical analyses showed that there was highly significant (P<0.01) effect of seasons and environmental conditions (Temperature & relative humidity) on the availability and amount of infective larvae on the pasture.

Fig. 1. Monthly mean of temperature, relative humidity and total rainfall**Fig. 2. Monthly mean of pasture larval counts of gastrointestinal nematode species recovered from herbage****Fig. 3. Monthly total pasture larval counts of gastrointestinal nematodes recovered from herbage**

DISCUSSION

The infective larvae or free-living stages of *H. contortus* were most prevalent on pasture of investigated area. Singh *et al.* (1997) and O'Connor *et al.* (2006) demonstrated that the monthly mean 50 mm of total rainfall and 18.3–34°C temperature provided optimum condition for the development and survival of free-living stages of *H. contortus*. The climatic data in the study area indicated that the required temperature for development persisted through out the year (Fig. 1). However required rainfall occurred between August and September. Therefore, it was expected that the higher number of free-living stages of *H. contortus* during these months. Furthermore, *H. contortus* parasite has

relatively short generation interval and ability to take the advantage of favorable environment conditions. Surprisingly, *Trichostrongylus* spp. and *Ostertagia circumcincta* larvae were more prevalent species in study area. These parasites considered dominant parasite species of temperate geographical regions where rainfall is uniform. The free-living stages of *Trichostrongylus* spp. and *Ostertagia circumcincta* species required total monthly rainfall over 50 mm and mean monthly maximum temperature of 12.8–18.3°C to develop and survive (Singh *et al.*, 1997; O'Connor *et al.*, 2006). The climatic data of study area indicated that adequate rains occurred during August and October when the prevalence of *Trichostrongylus* spp. reached the peak. This condition might be favorable for larval development of *Trichostrongylus* spp. in study area during these months, but the mean maximum temperature was over optimum temperature for development of these species. This could be attributed to emergence of new strains of gastrointestinal nematode adapted environmental conditions of subtropical humid regions. Furthermore, the eggs and infective larvae of *Trichostrongylus* spp. have been reported to have a high ability to survive under adverse weather condition (Urquhart *et al.*, 1996).

The highest prevalence of *O. circumcincta* larvae was in March, whereas the lowest was recorded in December. The results of current study are not supported by climatic data prevalent in study area. Fernandez *et al.* (2001) reported that the eggs of *O. ostertagia* of cattle reached to infective larvae stages in the pat faeces irrespective of environmental conditions, because the moisture in pat alone is sufficient for such development, in the light of findings of above worker the highest prevalence of *O. circumcincta* in March could be explained. The prevalence of *S. papillosus* larvae reached the highest level in August, whereas it declined to the lowest level in May. Urquhart *et al.* (1996) and Cheah and Rajamanickam (1997) suggested that the optimal temperature for the development of maximum number of larvae in the shorter feasible time is generally in the range of 18–26°C. At the higher temperature, development is faster and the larvae are hyperactive, thus depleting their lipid reserved. The mortality rate then rises, so that few will survive to infective larvae. The results of current study are contrary to the suggestions of above workers. The high prevalence of *S. papillosus* in August could be attributed to localized contamination of watering and feeding resources with free-living stages of *S. papillosus* during summer rainy season rendering the environmental condition favorable for development of these larvae in study area. Low number infective larvae of *S. papillosus* in May may be due to high environmental temperature in this month. Other, infective larvae of gastrointestinal nematodes recovered from herbage samples were, *Oe. Columbianum* and *Chabertia ovina* on varying percentages. The low number of free-living stage larvae of *Oe. Columbianum* and *Chabertia ovina* on pasture may be due to that the females of those parasites laid less number of

eggs, longer life-cycle or free-living stages have relatively short survival time on pasture compared to other gastrointestinal nematodes of sheep.

In general, the pasture larval counts were higher from February to October with peak of infectivity in August (summer season) then gradually declined from November onward to reach the minimum level in January (Fig. 2 & 3). The results of this study are in complete agreement with previously studies (Suarez & Busetti, 1995; Romjali *et al.*, 1997; Waruiru *et al.*, 1998). The most favorable condition for the development of pre-infective larval stages are fairly high temperature to ensure a rapid rate of development and adequate moisture to ensure faeces not become desiccated before the free-living larvae reached to infective stage and translocated to herbage (Cheah & Rajamanickam, 1997; Nginyi *et al.*, 2001). The subtropical humid environment of study area provides such condition for development of infective larvae of gastrointestinal nematodes of sheep. Furthermore, in sub-continent, the monsoon set in July and ends in September, with the highest rainfall in August, this could be made the environmental conditions more favorable for the development and survival of the pre-parasitic stages leading to increased availability of infective larvae on the pasture during monsoon and subsequent months (Singh *et al.*, 1997; Jithendran & Bhat, 1999; Iqbal *et al.*, 2005a), as noted in this study. A low number and availability infective larvae during the dry winter season (December-January) could be due to the high mortality of free-living stages as a result of the herbage cover was very low due to overgrazing, thus exposed the larvae to desiccation and resulting in high mortality or migration deeper into the soil (Ng'ang'a *et al.*, 2004).

The amounts of infective larvae are considered as epidemiological variable of high influence on the parasitic rates of animals under grazing condition (Krecek & Maingi, 2004). In study area, sheep and goats bred throughout the year and lambing and kidding seasons reached the peak during March-April and October-November. This might be increased contamination of pasture with high number of eggs shed by lactating ewes and does. The results of this study are in accordance with observations of Tembely *et al.* (1997), Cheah and Rajamanickam (1997), Jithendran and Bhat (1999), Waruiru *et al.* (2001) and Garcia *et al.* (2006), who conducted studies on epidemiology of gastrointestinal nematodes of sheep.

CONCLUSION

Pasture grazed by sheep at communal grazing pasture of study area remained contaminated with variety of free-living stages or infective larvae of gastrointestinal nematodes throughout the year. The humid subtropical environment of the study area provided favorable condition for the development and survival of free-living larvae of gastrointestinal nematodes. These results provide a tool for predicting pasture larval infectivity, which may be useful in designing control program for gastrointestinal nematodes for irrigated agro-ecological zones.

REFERENCES

- Cheah, T.S. and C. Rajamanickam, 1997. The epidemiology of gastrointestinal nematodes of sheep in wet tropical conditions in Malaysia. *Trop. Anim. Hlth. Prod.*, 29: 165–73
- Fernandez, S., M. Sarkunas and A. Roepstorff, 2001. Survival of infective *Ostertagia ostertagi* on pasture plots under different simulated grazing conditions. *Vet. Parasitol.*, 96: 291–9
- Garcia, J.A., J.G.R. Diego, G.T. Hernandez, M. Mahieu, E.G.G. Garcia and R. Garduno, 2007. The epizootiology of ovine gastrointestinal Strongyles in province of Matanzas. *Vet. Parasitol.*, 72: 119–226
- Gibson, T.E. and G. Everett, 1976. The effect of weather in modifying the patterns of pasture larval contamination with *Ostertagia circumcincta*. *Int. J. Biometeorol.*, 20: 49–55
- Gibson, T.E. and G. Everett, 1977. The contribution of over wintering pasture larval infection and spring rise as source of *Ostertagia circumcincta* infection in lamb. *Eas. Vet. Sci.*, 23: 191–5
- Hansen, J. and B. Perry, 1994. *The Epidemiology Diagnosis and Control of Helminth Parasites of Ruminants*. International laboratory for research on animal diseases, Nairobi, Kenya
- Iqbal, Z., M. Akhter, M.N. Khan and M. Ziad, 1993. Prevalence and economic significance of haemonchosis in sheep and goats slaughtered at Faisalabad abattoir. *Pakistan J. Agric. Sci.*, 30: 51–3
- Iqbal, Z., M. Lateef, M.N. Khan, G.M. Muhammad and A. Jabbar, 2005a. Temporal density of Trichostrongyloid larvae on a communal pasture in a subtropical region of Pakistan. *Pakistan Vet. J.*, 25: 87–91
- Iqbal, Z., A. Jabbar, M.S. Akhter, G. Muhammad and M. Lateef, 2005b. Possible Role of Ethnoveterinary Medicine in Poverty Reduction in Pakistan: Use of Botanical Anthelmintic as an Example. *Agric. Soc. Sci.*, 1: 188–95
- Jithendran, K.P. and T.K. Bhat, 1999. Epidemiology of parasitoses in dairy animals in the north-west humid Himalayan region of India with particular reference to gastrointestinal nematodes. *Trop. Anim. Hlth. Prod.*, 31: 205–14
- Krecek, R. and C.N. Maingi, 2004. Comparison of two techniques used for the recovery of third-stage Strongyloid nematode larvae from herbage. *Vet. Parasitol.*, 122: 233–4
- Lateef, M., 2002. Trichostrongyloid nematodes of sheep: Epidemiological aspects and evaluation of anthelmintic activity of indigenous plants. *Ph. D. Thesis*, University of Agriculture, Faisalabad, Pakistan
- MAFF, 1986. *Manual of Veterinary Parasitological Laboratory Techniques*, pp: 31–3. London, UK
- Mohiuddin, A., M.M. Khan, M.A. Shaikh and F.A. Mughal, 1984. Taxonomy, incidence and seasonal variation of helminth parasites of sheep and goats of Sindh. *Pakistan J. Zool.*, 16: 25–30
- Ng'ang'a, C.L., N. Maingi, P.W.N. Kanyari and W.K. Munyua, 2004. Development, Survival and Availability of Sheep on Pasture in a semi-arid Area of Kajiado District of Kenya. *Vet. Res. Commun.*, 28: 491–501
- Nginyi, J.M., J.L. Duncan, D.J. Mellor, M.J. Stear, S.W. Wanyangu, R.K. Bain and P.M. Gatongi, 2001. Epidemiology of parasitic gastrointestinal nematode infections of ruminants on smallholder farms in central Kenya. *Res. Vet. Sci.*, 70: 33–9
- O'Connor, L., W. Stephen and W.W. Brown, 2006. Ecology of the free-living stages of major Trichostrongyloid parasites of sheep. *Vet. Parasitol.*, 142: 1–15
- Pal, R.A. and M. Qayyum, 1992. Breed, age, sex-wise distribution of gastrointestinal helminths of sheep and goats in and around Rawalpindi region. *Pakistan Vet. J.*, 12: 60–3
- Rinehart, L., 2006. *Pasture, Rangeland and Grazing Management*. ATTRA, National Sustainable Agriculture Information, USA
- Romjali, E., P. Dory, A. Batubara, V.S.P. Andey and M. Gatenby, 1997. Peri-parturient rise in faecal Strongyle egg counts of different genotypes of sheep in North Sumatra, Indonesia. *Vet. Parasitol.*, 68: 191–6
- Singh, D., C.P. Swarnkar, F.A. Khan, C.P. Srivastava and P.S.K. Bhagwan, 1997. Epidemiology of ovine gastrointestinal nematodes at an organized farm in Rajasthan, India. *Small Rumin. Res.*, 26: 31–7

- Suarez, V.H. and M.R. Buseti, 1995. The epidemiology of helminth infection of growing sheep in Argentina's Western Pampas, *Int. J. Parasitol.*, 25: 489–94
- Tembely, S., A.L. Kassi, J.E. Rege, S. Sovani, M.L. Diedhiou and B.L. Baker, 1997. The epidemiology of nematode infections in sheep in cool tropic environment. *Vet. Parasitol.*, 70: 129–241
- Urquhart, G.M., J. Armour, J.L. Dunncan and F.W. Jennings, 1996. *Veterinary Parasitology*, 1st edition. Longman, London, UK
- Waruiru, R.M., W.K. Munyua, S.M. Thamsborg, P. Nansen, H.O. Bogh and J.M. Gathuma, 1998. Development and survival of infective larvae of gastrointestinal nematodes of cattle on pasture in central Kenya. *Vet. Res. Commun.*, 22: 315–34
- Waruiru, R.M., S.M. Thamsborg, P. Nansen, N.C. Kyvsgaard, H.O. Bogh, W.K. Munyua and J.M. Gathuma, 2001. The epidemiology of gastro-intestinal nematodes of dairy cattle in central Kenya. *Trop. Anim. Hlth. Prod.*, 33: 173–87

(Received 02 August 2008; Accepted 29 August 2008)