Studies on abomasal nematodes of sheep in temperate climatic conditions of Kashmir, India

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Abstract: An epidemiological study with an objective to assess the seasonal dynamics and over winter survival of abomasal nematodes of sheep in a temperate climate of Kashmir valley, India was carried out in naturally infected sheep over a period of two years. Faecal examination [prevalence (%age) and faecal egg counts (epg), larval differentiation] and necropsy of the study animals were the methods employed. *Haemonchus contortus* and *Teladorsagia* (*Ostertagia*) *circumcincta* were most prevalent followed by *Trichostrongylus* sp. however; the prevalence differed (non significant) in the two years. Summer showed significantly higher prevalence of all the three parasites as compared to other seasons. The faecal egg counts and necropsy observations revealed arrested development (hypobiosis) within the host body during winter (P = 0.008), which was confirmed through the recovery of L4 and adult nematodes from host abomasums in winter. The present study indicates the seasonality and arrested development of abomasal nematodes in the sheep under the temperate climatic conditions and validates their strategic overwinter survival and resumption of development on the onset of suitable environmental conditions in spring.

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1. Introduction

Problems with nematode infections of sheep have increased in recent years and causes severe damage to sheep in terms of high mortality, reduced body weight, wool yield and birth rate (Ahmad, 1992). Sheep are more prone to nematode infection as they mostly graze on pastures contaminated with infective larval forms. Most infections are mixed infections and pathogenicity is usually high when *Haemonchus* and *Trichostrongylus* occur together in sheep. According to Fox (1993), haemonchosis and ostertagiosis can result in accentuated economic losses by causing appetite, depression, damage in gastric function, and alteration in total protein content, energy and mineral metabolism.

Arrested development or hypobiosis is a well known phenomenon in abomasal nematodes of ruminants (Gibbs, 1986; Eysker, 1993). Hypobiosis is a mechanism whereby the parasite avoids shedding eggs into external environment at times of the year when the surrounding conditions are unfavourable to its development, thus avoiding mortality of its free living stages. In spite of the fact, that sheep has the greatest importance in the animal resources of Kashmir; no concrete reports are available on the epidemiology and management of their abomasal nematodes (Tariq *et al.,* 2008; Dhar *et al.,* 1982). The present study was aimed at to investigate the seasonal prevalence of infection with naturally occurring abomasal nematodes of sheep as well as to ascertain the occurrence of the phenomenon of their over winter survival during the chilling and snowy winter in the temperate climatic conditions of Kashmir valley, India.

2. Materials and methods

2.1. Study area

Kashmir valley lies between 33˚-20′ and 34˚-54′ North latitude and 73˚-55′ and 75˚-35′ East longitude covering an area of 15,948 sq. km. The temperature ranges from an average daily maximum of 31˚C and minimum of 15˚C in June-July to an average daily maximum of 4˚C and minimum of - 4˚C in January. The average rainfall at Srinagar is 659 mm/anum, and most of the precipitation occurs in the form of snow during winter. An average of 600 mm of snowfall occurs in Srinagar during winter and early spring, but the snowfalls on the higher slopes are much heavier. On the basis of temperature and precipitation, four seasons in a year recognised in the valley are: Winter (December to February); Spring (March to May); Summer (June to August); Autumn (September to

November). Three well defined systems of traditional sheep rearing in Kashmir valley are migratory, semi-migratory and sedentary. Forages are the major sources of feed for sheep sustenance in this region, which are produced on pastures, meadows and on cultivable lands.

2.2. Animals

The study is a part of a PhD thesis and was conducted over a period of two years on a sheep flock in Kashmir valley, India. A total of 30 sheep (*Ovis aries* Linn.) were examined to obtain data on prevalence, seasonal variations and the possible over winter survival of the abomasal nematodes. The animals were of both sexes up to 4 years of age and belonged to Local Kashmiri, Corriedale, and Cross bred local (Kashmiri × Corriedale) breeds.

2.3. Grazing systems

The sheep were managed under a traditional system, grazed on pastures and fallow lands during late spring, summer and early autumn months together with animals owned by neighboring small-holder farmers. The grazing areas consisted of alpine, sub alpine and communal grazing areas shared by many flocks. The animals were housed at night in wooden fences nearby to pastures. During the cold and snowy winter the animals were housed in the pens and fed supplementary feeds consisting of dry *Salix* leaves, fodder, maize and pulses. The animals were not treated with any anthelmintic during the study period.

2.4. Collection and analysis of faecal samples

All the 30 animals in the flock were faecal sampled weekly and their egg output estimated in eggs per gram (epg) of faeces by the Stoll's method. The eggs were identified on the basis of morphological characters as given by Soulsby (1982). For Larval differentiation and identification of the parasites, the positive faecal samples were subjected to faecal culture once a month. Faeces were cultured in an incubator for 7 days at 26 – 27ºC to obtain L_3. The resulting L_3 were recovered from the coprocultures by Baermann Technique and identified on the basis of morphological keys as described by Soulsby (1982).

2.5. Necropsy

In the months of February during the course of two years, 5 sheep were selected randomly from the flock and slaughtered to examine the arrested nematodes. Larger number of animals could not be slaughtered due to economic reasons. As five animals were slaughtered so fresh five animals were added to the flock to maintain the required number of animals for the second year of the study. At necropsy, the abomasum was immediately removed and the contents were put in a plastic bucket. The mucosa was cleaned under clean running tap water and the washings were drained into bucket containing 5% formalin. The mucosa was scraped off the abomasum and the scrapings were digested in pepsin- Hcl mixture and preserved in 5 % formalin. The entire contents were observed under the Olympus Research Microscope for adult nematodes and arrested L_4. The worm counts were estimated per sheep slaughtered. This method was similar to that described earlier by Gatongi *et al.*, 1998).

2.6. Data analysis

Percentages to measure prevalence and chi-square to measure association between the parasitism and season were the statistical tools applied using Statistical packages MINITAB software version 13.2 (Minitab 2002). Faecal egg counts were expressed as eggs per gram of faeces. Confidence level was held at 95 % and $P < 0.05$ was set for significance.

3. Results

The present study on abomasal nematodes of sheep in temperate climate of Kashmir valley revealed the presence of *Haemonchus contortus, Teladorsagia* (*Ostertagia*) *circumcincta* and *Trichostrongylus* sp. with the former two most prevalent species among the three. In the second year of study the prevalence was comparatively less than the first year (Table 1). The results of faecal egg counts (epg) demonstrated seasonal dependence (Table 2) of infection with maximum infection in summer season ($P = 0.008$). The faecal egg counts and necropsy findings showed arrested development (hypobiosis) within the host body during winter. When the temperature started increasing from March onwards it incidentally resulted in the increasing production of eggs by the parasites.

The monthly prevalence of different parasites (Table 3) and the necropsy observations (Table 4) revealed the presence of L_4 larvae and adults in the dormant state in the abomasum of the sheep. Among the observed parasites *H. contortus* was present consistently in the abomasum of all the examined sheep. The same animals faecal examined were devoid of eggs in their faeces. This indicated that nematodes had arrested their development and could not produce eggs. For every month assayed during the course of study, the proportion of the hypobiotic (inhibited) nematodes eventually declined abruptly to minimum levels as observed through the increase in egg counts from March onwards. This indicated adults have resumed development and produced eggs detectable in the sheep faecal matter.

4. Discussion

The seasonal activity of abomasal nematodes in the present study appeared to be influenced by climatic conditions and in conformity with the findings of other authors (Eysker, 1997; Vlasoff *et al.*, 2001; Khajuria and Kapoor, 2003; Lateef *et al.*, 2005; Sreedevi and Murthy, 2005; Umur and Yukari, 2005; Bersissa and Abebe, 2006; Qamar et al., 2009). Translation of the infective stages was slow on pastures earlier on, but as rainfall, temperature, and vegetative ground cover increased towards mid summer (June-July), transmission of the infective stages occurred with increasing frequency. It is well documented that gastrointestinal nematodiasis in grazing animals is directly related to the availability of larvae on pasture and seasonal pasture contamination (Soulsby, 1982, Garedaghi and Bahavarnia, 2013). It has been earlier indicated that rainfall distribution was the major factor governing the development, distribution, dissemination and survival of free living stages of nematodes on pastures (Ng'ang'a *et al* 2004).

The low epg reported during the winter season in the present study could be attributed to low temperature which helps in hypobiosis in host. The low faecal egg counts (epg) in winter and the presence of L_4 as arrested larval stages in the abomasum confirmed the established view that abomasal nematodes in sheep can successfully over winter in temperate climates characterized with falling temperature or chilling in winter (Gibbs, 1986). This presents an interesting example of an ecological adaptation of *H. contortus*, *T. circumcincta* and *Trichostrongylus* sp. to their local climatic conditions and have evolved to survive the long, cold harsh winters of the valley entirely within the host as arrested larval stage (L_4), relying on the onset of favourable environmental conditions of temperature, which triggered the resumption of development to the adult egg laying parasites. The presence of snow cover in the external environment was one explanation behind this observation as has been reported earlier in other parts of the world (Ng'ang'a *et al.*, 2004). It is a well established fact that the advent of adverse climate conditions impairs the survival of parasites on pasture (Smeal and Donald, 1984; Boag and Thomas, 1985; Nabavi *et al.*, 2011) and the parasites respond to the stimulus by arresting their development at an early larval stage and wait until the opportunity for transmission to another host is available (Lutzelschwab *et al*., 2005).

Hypobiosis and its epidemiological significance have been well documented in parasitic nematodes of ruminants (Vercruysse, 1985; Fiel *et al.*, 1988; Sargison *et al.*, 2007; Nabavi *et al.*, 2011). In the winter, parasites did not find the optimum environmental conditions to disseminate their eggs and the larvae could not develop due to very low temperature, which fell to -4ºC particularly in January. Temperature also influences the development of nematode larvae and the optimum temperature for the development of most trichostrongylid larvae is 22-30ºC. No development of trichostrongylid larvae occurs below 5ºC while temperatures above 40ºC are lethal. Thus a special ecological mechanism had come into existence in abomasal parasites in the temperate climate to ensure the over winter survival within the host body. Whether this hypobiotic profile of abomasal nematodes of sheep in temperate climate of Kashmir valley is a recent adaptation, or not, remains unanswered. However, further experimental studies are needed to confirm the findings of this study.

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Table 1. Prevalence of abomasal nematodes in sheep over the period of two years

Parasites	Year	Hosts Examined	Infected (%)
Haemonchus contortus	Year 1	30	21 (70.0)
	Year 2	30	20 (66.6)
Teladorsagia circumcincta	Year 1	30	14 (46.6)
	Year 2	30	13 (43.3)
Trichostrongylus sp.	Year 1	30	10 (33.3)
	Year 2	30	9 (30.0)

Table 2. Mean ± Standard error of mean (SE) of eggs per gram of faeces (epg) of sheep in different seasons over the period of two years (P = 0.008)

Season	Mean ± SE of epg of faeces	
	Year Ist	Year IInd
Winter season (December, January, February)	11. 6 ± 11.6 (35,0,0)	10.0 ± 10.0 (30,0,0)
Spring season (March, April, May)	537. 3 ± 75.1 (410, 532, 670)	420.0 ± 47.7 (326, 452, 482)
Summer season (June, July, August)	891. 0 ± 45.5 (800, 938, 935)	843.3 ± 49.8 (744, 886, 900)
Autumn season (September, October, November)	700.0 ± 163.7 (920, 800, 380)	666. 6 ± 180.2 (890, 800, 310)

Table 3. Monthly occurrences of abomasal nematodes in the faecal samples of sheep over the period of two years

Year Ist			
Month	No. Examined	No. Positive	Nematodes
January	30	---	---
February	30	---	---
March	30	09	*Haemonchus, Teladorsagia*
April	30	17	*Haemonchus, Teladorsagia,*
May	30	21	*Teladorsagia, Trichostrongylus*
June	30	22	*Haemonchus,Teladorsagia, Trichostrongylus*
July	30	24	*Haemonchus,Teladorsagia, Trichostrongylus*
August	30	23	*Haemonchus, Trichostrongylus*
September	30	19	*Haemonchus,Teladorsagia, Trichostrongylus*
October	30	16	*Haemonchus,Teladorsagia, Trichostrongylus*
November	30	11	*Haemonchus, Teladorsagia,Trichostrongylus*
December	30	05	*Haemonchus*
Year IInd			
January	30	---	---
February	30	---	---
March	30	08	*Haemonchus*
April	30	16	*Haemonchus, Teladorsagia*
May	30	19	*Haemonchus, Teladorsagia,*
June	30	19	*Haemonchus, Teladorsagia, Trichostrongylus*
July	30	21	*Haemonchus, Teladorsagia, Trichostrongylus*
August	30	19	*Haemonchus, Teladorsagia, Trichostrongylus*
September	30	17	*Teladorsagia, Trichostrongylus*
October	30	12	*Haemonchus,Teladorsagia, Trichostrongylus*
November	30	09	*Haemonchus,Teladorsagia, Trichostrongylus*
December	30	06	*Haemonchus, Trichostrongylus*

Table 4. Necropsy results for five sheep slaughtered among the experimental flock to confirm the occurrence of hypobiosis

Year Ist					
	Sheep 1	Sheep 2	Sheep 3	Sheep 4	Sheep 5
Parasites	*Haemonchus* (68)	*Trichostrongylus* (42) *Teladorsagia* (54)	*Haemonchus* (70)	*Haemonchus* (61) *Teladorsagia* (49) *Trichostrongylus* (42)	*Haemonchus* (77) *Teladorsagia* (60)
Year IInd					
	Sheep 1	Sheep 2	Sheep 3	Sheep 4	Sheep 5
Parasites	*Haemonchus* (57) *Teladorsagia* (40)	*Haemonchus* (66) *Trichostrongylus* (60)	*Haemonchus* (69) *Teladorsagia* (68) *Trichostrongylus* (60)	---	*Haemonchus* (73)

Figures in parentheses indicate total worm burden

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