



Research Article

Prevalence and *in Vitro* Culture of *Trichostrongylus* Spp. in Goat at Trishal, Mymensingh, Bangladesh

Sheik Mohammad Fazly Rabby Rajib, Anita Rani Dey*, Nurjahan Begum, Mohammad Abdul Momin, Mohammad Hasanuzzaman Talukder

Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202
*Corresponding author: anitadey.dpp.vet@gmail.com

ARTICLE HISTORY

Received: 2014-08-10
Revised: 2014-11-01
Accepted: 2014-11-04

Key Words: Prevalence, In vitro, culture, *Trichostrongylus* spp., Goat

ABSTRACT

Trichostrongylus spp., Looss, 1905 is one of the important nematode of alimentary canal that causes a great harm to the ruminants. The study was undertaken to determine the prevalence of *Trichostrongylus* spp. and effects of temperature, pH, humidity, light and dark, nutrient and other media on the hatching of eggs, the development and survival of larvae of *Trichostrongylus* spp. Samples were collected from goats from different villages and slaughter houses of Trishal, Mymensingh, Bangladesh, during the period from January to May, 2014. A total of 82 fecal and 67 visceral samples (abomasum, small intestine and large intestine) were collected. Overall prevalence was 20.73% and 14.93% for fecal samples and visceral samples, respectively. Young (23.64%) were 1.78 times more susceptible to *Trichostrongylus* infection than adult (14.82%) and which was statistically significant ($p < 0.001$). It was also revealed that females (23.08%) were 1.31 times more susceptible than male (18.61%) and statistically significant ($p < 0.001$). Here, we incubated eggs at different temperature gradients, humidity and pH in phosphate buffer saline (PBS), tap water (TP) and normal saline (NS) and PBS with 5%, 10% and 15% serum. Maximum (46.67%) hatching of eggs was observed at 18°C on day 5. and development of eggs arrested at 4°C temperature. No development and hatching occurred at pH 2. Hatching of eggs were better on day 6 in all cases and highest hatching rate observed at pH 6.0 (32.30%). Maximum hatching of eggs (42.86%) was recorded at relative humidity of >80%–90% at day 5. There was no significant effect of light on the development and hatching of eggs of *Trichostrongylus* spp. Maximum eggs hatched in PBS containing 15% serum (46.67%). Moreover, in this medium, survival rate of larvae was also highest (19.08%). So, it is concluded that PBS containing 15% serum can be used as a suitable media for hatching of eggs and survival of larvae.

All copyrights reserved to Nexus® academic publishers

ARTICLE CITATION: Rajib SMFR, Dey AR, Begum N, Momin MA, Talukder MH (2014). Prevalence and in vitro culture of *Trichostrongylus* spp. in goat at Trishal, Mymensingh, Bangladesh. J. Adv. Parasitol. 1 (4): 44 – 48.

INTRODUCTION

Bangladesh is a developing country where goat ranks second in meat, milk, and skin production, representing about 28%, 23% and 28% respectively of the total livestock in Bangladesh (Banglapedia /goat/ 2013). But the productions are hindered by various types of infection. Among them parasitic infection is most common. Geographical location and agro-ecological condition in Bangladesh are highly conducive to the growth and multiplication of a large number of parasite species. *Trichostrongylus* spp. is one of the important nematode of alimentary canal that causes a great harm to the ruminants. Nematodes especially *Trichostrongylus* spp. is prevalent worldwide (Dhar et al., 1982; Ha and Vu, 2013; Rehbein et al., 2012). *Trichostrongylus* spp. is one of the common, pathogenic and also prevalent nematodes recorded in Bangladesh (Haq and Shaikh, 1968). The development, distribution, or migratory behavior of the free living stages (i.e. eggs, developing and infective larvae) of gastro-intestinal nematodes on pasture is primarily weather related. Environmental factors influenced both the

development and survival of the larvae on pasture and their distribution on the herbage. In the pasture, deposited eggs hatch and develop to the infective L3 stage. Under optimal conditions as moisture and temperature, larvae of *Trichostrongylus* spp. reach the infective stage in approximately 5–6 days. Developmental time depends on environmental condition. Eggs hatch and develop more slowly at lower temperatures. The rate of development increases to a maximum at higher temperatures after which development will be adversely affected and death of the larvae occurs (Soulsby, 1982). The primary factors that affect the hatching of eggs, development and survival larvae are temperature and moisture and vary according to the parasite. *In vitro* information about effects of temperature, pH, humidity, light and nutrients on the hatching of eggs and survival of larvae of *Trichostrongylus* spp. are essential to study the biology of the parasite. By considering these facts, the present study has been designed to determine the prevalence and to investigate the effects of temperature, pH,

nutrients, humidity and light on the hatching of eggs and survival of larvae of *Trichostrongylus* spp.

MATERIALS AND METHODS

Study Area

The study was carried out in different villages and slaughter houses of Trishal in Mymensingh, Bangladesh. Identification, hatching and observation of larvae were performed in the laboratory, Department of Parasitology, Bangladesh Agricultural University, Mymensingh.

Study Period

The study was conducted from January to May, 2014.

Selection of Goats

Goats (82) were selected randomly. The age of the goats were determined by examining the teeth (Samad, 2008). According to the age, goats were divided into two groups such as < 1.5 years (55) and >1.5 years (27) of age. The sex of the goats was also recorded before slaughter.

Collection of Fecal Sample

Eighty two (82) fecal samples were collected directly from the rectum of goats. After collection, samples were placed in a polythene bag and packed tightly by mixing with 10% formalin and brought to laboratory.

Collection of Visceral Sample

Sixty seven (67) visceral samples (abomasum, small and large intestine) were collected. After collection, sample was packed in a plastic bag and brought to laboratory. Collections of parasites from intestine were made following the standard procedure described by Rahman (1969). Identification of species with sex differentiation was based on the morphology as described by Soulsby (1982).

Recovery of Eggs

Matured eggs were recovered directly from the ovipositing female (*Trichostrongylus* spp.). For this, female parasites were crushed by using sterile mortar and pestle containing necessary amount of phosphate buffer solution (PBS). Debris was picked up with needles and the contents were transferred to a clear Petri dish through sieving. The suspension was gently stirred to make uniform solution. Eggs were counted by McMaster technique (Soulsby, 1982).

Effects of Temperature

To study the effects of temperature, the pre-counted numbers of eggs were suspended in a Petri dish containing culture media and incubated at various temperatures (37°C, 27°C, 18°C, 8°C and 4°C) up to 8 days. Identification of different stages of larvae was made on the basis of morphological descriptions described by Anon (1977), Soulsby (1982) and Rahman et al., (1996).

Effects of pH

To study the effects of pH, the different pH levels (2.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) of the culture media were adjusted by adding glacial acetic acid or by adding Sodium hydroxide in PBS drop by drop with the help of dropper and stirred the media. The pH was detected by pH meter. Pre-counted eggs were mixed in the culture media having above mentioned pH levels and kept in room temperature. Observations were

made by dissection microscope in every 24 hours up to 7 days.

Effects of Humidity

To determine the effects of relative humidity, the pre-counted number of eggs were mixed in the PBS and incubated at 70% – <80% and >80% – 90% of relative humidity at constant 26°C temperature. Developmental stages were observed by dissection microscope for every 24 hours up to 7 days.

Effects of Light

To study the effect of light, pre-counted number of eggs counting culture media was covered with hard paper to make dark condition having facilities for air exchange keeping in room temperature. Control media was also kept in room temperature at light condition. Then observation was made in similar manner up to 8 days.

Effects of Different Media

To find out a suitable media for the cultivation of eggs, pre-counted eggs were cultured in phosphate buffer solution (PBS), normal saline (NS) and tap water (TW) and incubated at room temperature (26°C) and pH 6.5 in 80–95% Relative humidity and observed in similar procedure.

Effects of Different Nutrients

For the preparation of different nutrient media, 5%, 10%, 15% serum of goat was added separately to the PBS. A pre-counted number of eggs were suspended in each and every culture medium mentioned above and all were incubated at room temperature up to 8 days. Then the percentage of hatching eggs was calculated for every 24 hours.

Statistical Analysis

Student t-test was used to determine the significance ($p < 0.05$) among the different variables (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Prevalence of *Trichostrongylus* Spp. In Goats

In the present study, overall prevalence of *Trichostrongylus* spp. in fecal sample and visceral sample were 20.73% (17/82) and 14.93% (10/67), respectively (Figure 1). More or less similar finding in fecal sample survey was also observed by Ha and Vu (2013). But the present study differs with Achi et al., (2003); Abebe and Esayas (2001) and Patel et al., (2001) who observed the prevalence rate were 46%, 64.3% and 9.2%, respectively.

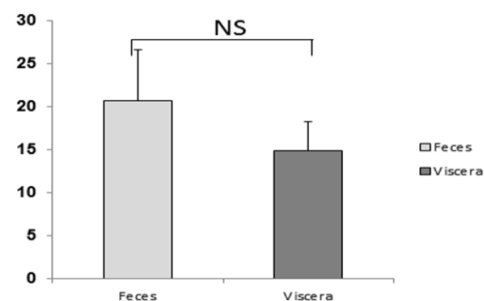


Figure 1: Over all prevalence of *trichostrongylus* spp. in goats

Females (23.08%) were 1.31 times more susceptible than males (18.61%) and it was statistically significant ($p < 0.001$) (Figure 2). The exact cause of higher rate of infection of *Trichostrongylus* spp. cannot be explained, but in general, higher level of prolactin and progesterone hormone suppress the immune system of the individual and make the individual more susceptible to any infection (Lloyd,1983).

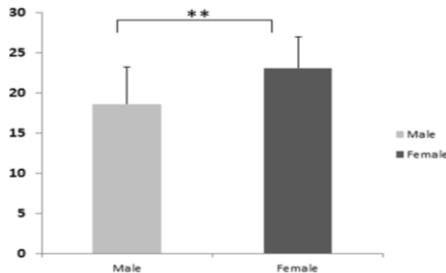


Figure 2: Sex related prevalence of *Trichostrongylus* spp. in goats

During this study, it was also recorded that young (23.64%) were 1.78 times more prone to *Trichostrongylus* spp. infection than adult (14.82%) and it was statistically significant ($p < .001$) (Figure 3). This may be due to low level of immunity in young animals.

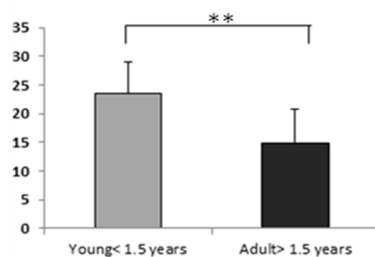


Figure 3: Age related prevalence of *Trichostrongylus* spp. in goats

In Vitro Culture of *Trichostrongylus* Spp. in Goats

Maximum Eggs were hatched at 18°C Temperature
 In the present study, developments of eggs were arrested at 4°C temperature and failed to develop even when returned to room temperature (15–20°C) during the period of observations up to 15 days. Better results were observed at 37°C temperature but maximum (46.67%) hatching of eggs were at 18°C on day 5. Details were shown in Table 1.

Table 2: Effects of pH on the hatching of eggs of *Trichostrongylus* spp.

During this study, hatching of eggs did not occur at pH 2.0. Sommerville and Murphy (1983) support this observation and interpret as it might be due to coagulation of germinal mass of the eggs due to high acidic pH level of the media. Ashad et al., (2011) and Stringfellow (1986) also found that

Table 1: Effects of Temperature on the hatching of eggs of *Trichostrongylus* spp.

Temperature (°C)	Hatching of Egg (%)				
	Day 1	Day 2	Day 3	Day 4	Day 5
4 (N=10)	–	–	–	–	–
8 (N=18)	–	–	11.11	16.67	22.23 ^a
18 (N=15)	–	13.33 ^a	20.00	33.33	46.67 ^b
27 (N=21)	–	4.76 ^a	14.28	23.81	38.09 ^b
37 (N=16)	–	18.75 ^a	31.25	37.50	43.75 ^{ab}

Values in the same column having different superscript are statistically significant ($p < 0.05$); N= Number of eggs

In the present study, No development were observed at low temperature (4°C). Ross and Small (1980) and Tripathi (1980) had similar observation. The exact mechanism of inactivation of eggs at lower temperature is not known but may be due to cold injury which squeezed the germinal mass and the eggs were devitalized. At 8°C, hatching of eggs started at day 3 which was relatively delayed than in other temperature. This finding was in concordance the findings of Soulsby (1982) who recorded that low temperature retarded the development of eggs and little development took place below 9°C. In the present study, maximum hatching was observed at 18°C on day 5. Minato et al., (2008) found that 15°C temperature was shown to favor direct development, producing infective larvae. Islam and Ahmed (1987) observed that eggs hatched at 15–34°C in 6 days which support the present study. At 27°C temperature, 4.76% eggs hatched on day 2 and maximum 38.09% of eggs hatched at day 5 and larvae attained infective stage at day 5. Whereas Rahman et al., (1996) found that eggs hatched within 1–2 days at 26°C and reached at the infective stage within 3–4 days. Similar observations were made by Tripathi (1977) where he mentioned that 20°C –30°C was the most suitable temperature for hatching and development of *Trichostrongylus* spp.

pH 6.0 is Optimum for Development and Hatching of *Trichostrongylus* Spp.

In this study, development and hatching of eggs was not observed in pH 2.0. Hatching of eggs was initiated from day 2 only in pH 6.0 and pH 7.0. Highest hatching was observed at pH 6.0 (32.30%) and lowest was at pH 4.0 (5.17%) at day 6 and day 7, respectively (Table 2).

Observations	Effect of different pH					
	2.0	4.0	5.0	6.0	7.0	9.0
Day 1	–	–	–	–	–	–
Day 2	–	–	–	12.00	7.98	–
Day 3	–	6.12 ^a	14.50 ^b	18.34 ^a	17.38 ^a	14.70 ^b
Day 4	–	8.67 ^a	18.26 ^b	25.56 ^a	21.78 ^a	14.88 ^{ab}
Day 5	–	14.78 ^a	22.30 ^b	28.37 ^a	29.90 ^a	15.44 ^b
Day 6	–	19.60 ^a	24.49 ^b	32.30 ^a	29.19 ^a	18.34 ^b
Day 7	–	5.17 ^{ab}	5.90 ^a	14.80 ^a	17.03 ^a	10.17 ^{ab}

Values in the same row having different superscript are statistically significant ($p < 0.05$).

maximum hatching of eggs and development of *Trichostrongylus* spp. larvae occurred at pH 6.0–7.0 which also support the present observation. Again developments were decreased at pH 9 (Table 2), which is possibly by the denaturation of egg mass in alkaline pH.

Favorable (>80–90%) RH is Necessary for Hatching of Eggs of *Trichostrongylus* Spp.

Maximum (42.86%) eggs were hatched at >80–90 relative humidity at day 5 and minimum (14.28%) were observed at 70–<80 relative humidity on day 3. Eggs were arrested in their development on day 1 and 2 in both cases (Table 3).

In this study, maximum (42.86%) eggs were hatched in case of relative humidity >80–90% which are less than the finding of Rahman et al., (1996), Rossanigo and Gruner (1994) and Berbigier et al., (1990) who found that optimum egg hatching and larval development and survival was 49.03%, 54% and 74%, respectively in the temperature range from 15°C to 20°C and at humidity from 79.5 to 95.5%.

Dark Condition is Favorable for Hatching of Eggs

Dark and light had no significant effect on development and hatching of eggs of *Trichostrongylus* spp. It was observed that no eggs were developed on first two days in both cases. But

developments were better in dark than light in all the observed day (Table 3). Islam and Ahmed (1987) and Ashad et al., (2011) also found that dark condition was more effective than light on the hatchability of eggs of *Trichostrongylus* spp.

Maximum Hatchability of Eggs and Survivability of Larvae was Observed when Grown in PBS

Hatching of eggs was initiated on day 3 in all media such as Phosphate buffer saline (PBS), normal saline (NS) and tap water (TW). Initially, the rate of hatchability was relatively higher in PBS (16.67%) followed by TW (14.29%) and NS (7.10%). On three culture media, maximum hatchability was observed in PBS (38.89%) on day 5. It was found that 22.94% larvae survived in PBS whereas in NS and TW, 8.34% and 6.47% larvae survived, respectively on day 8 (Table 4).

Table 3: Effects of humidity, dark and light on the hatching of eggs of *Trichostrongylus* spp.

Day	Effect of different humidity (%)		Effect of light	
	70–<80 (N=21)	>80–90(N=21)	Dark (%) (N=16)	Light (%) (N=16)
Day 1	–	–	–	–
Day 2	–	–	–	–
Day 3	14.28 ^a	23.81 ^b	12.50	6.25
Day 4	23.81 ^a	38.09 ^b	25.00	12.50
Day 5	33.33 ^a	42.86 ^b	43.75	31.25
Day 6	38.09	–	18.75	12.50

Values in the same row having different superscript are statistically significant (p<0.05); N= number of eggs

Table 4: Effects of media on the hatching of eggs and survival of larvae of *Trichostrongylus* spp.

Media	No. of eggs	Observations (%)										
		Hatching of eggs					Survival of larvae					
		D1	D2	D3	D4	D5	D6 d	l	D7 d	l	D8 d	l
Normal Saline (NS)	14	–	–	7.10	14.30	28.57 ^a	7.14	21.42	10.42	16.15	19.23	8.34 ^a
Phosphate Buffer Solution (PBS)	18	–	–	16.67	27.77	38.89 ^b	7.00	30.99	10.34	24.08	12.18	22.94 ^b
Tap Water (TW)	21	–	–	14.29	19.04	28.57 ^{ab}	5.99	22.58	14.90	13.67	22.10	6.47 ^a

Values in the same row having different superscript are statistically significant (p<0.05); d= dead, l= live, D= day of experiment; N= number of eggs

Table 5: Effects of nutrients on the hatching of eggs and survival of larvae of *Trichostrongylus* spp.

Media	Observations (%)										
	Hatching of eggs					Survival of larvae					
	D1	D2	D3	D4	D5	D6 d	l	D7 d	l	D8 d	l
PBS+ 5% serum (N=20)	–	10.00	15.00	25.00	30.00 ^a	3.12	20.44	7.38	14.14	8.46	12.24 ^a
PBS+ 10% serum (N=15)	–	11.11	26.66	38.89	44.45 ^a	3.96	28.79	4.09	27.43	10.60	18.90 ^b
PBS+ 15% serum (N=18)	–	13.33	27.78	40.00	46.67 ^b	3.02	29.43	6.66	23.59	10.50	19.08 ^a

Values in the same row having different superscript are statistically significant (p<0.05); d= dead, l= live, D= day of experiment; N= number of eggs

During this study, hatchability and survivability was maximum in PBS. These results were clearly similar to the results obtained by Islam et al., (2005) who achieved the highest L3 yield in PBS (23.82%). In this study, No development were seen in first two days. This observation differs with Veglia (1916) who found that L1 hatched from eggs within 14–17 hours. The variation in development and hatching of eggs may be due to other condition applied.

Nutrient is Essential on the Hatching of Eggs and the Survival of the Larvae of *Trichostrongylus* Spp.

In this study, hatching was initiated from day 2 and gradually increased in all the media but best result was

found in PBS containing 15% serum which was initiated as 13.33% on day 2 and finally reached 46.67% on day 5. Death of larvae initiated on day 6 in all media; survival and longevity of larvae was maximum (19.08%) in PBS containing 15% serum (Table 5). PBS containing 15% serum is comparatively more enriched than other media. This enriched media ensured a balanced environment and provide protein and several vitamins. Paul (1965) supports the finding who recorded the increased survival of larvae in a uniform manner in a medium containing plentiful food source.

ACKNOWLEDGEMENTS

Respected teachers in the department of Parasitology for their kind help.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

- Abebe W, Esayas C (2001). Survey of ovine and caprine gastrointestinal helminthosis in eastern part of Ethiopia during the dry season of the year. *Rev. Med. Vet.* 152: 379 – 384.
- Achi YL, Zinsstag J, Yeo N, Dea V, Dorchie P (2003). Epidemiology of parasites of sheep and goats from savannah area in Cote d'Ivoire. *Rev. Med. Vet.* 154: 179 – 188.
- Anon (1977). *Manual of Veterinary Parasitology Laboratory Techniques*. 2nd edn. (HMSO) Her Majesty's stationary Office. Roben dene Amersham Ltd. England, pp. 14 – 23.
- Ashad FA, Anisuzzaman, Begum N, Dey AR, Mondal MMH (2011). Factors affecting the development and hatching of eggs and the survival of infective larvae of *Haemonchus contortus* in laboratory condition. *Progress. Agric.* 22(1 & 2): 75 – 83.
Banglapedia.htm/goat/2013
- Berbigier P, Gruner L, Mambrini M, Sophie SA (1990). Faecal water content and egg survival of goat gastro-intestinal strongyles under dry tropical conditions in Guadeloupe. *Parasitol. Res.* 76: 379 – 385.
- Dhar DN, Sarma RL, Bassal GC (1982). Gastro-intestinal nematodes in sheep in Kashmir. *Vet. Parasit.* 11: 273 – 277.
- Ha TH, Vu DD (2013). Observations on Parasitism of goats raised in Bavi district (Hatay province). *Khoa Hoc KyThuat Thu Y Veterinary Sciences and Techniques*. 10: 36 – 41.
- Haq S, Shaikh, H (1968). A survey of helminths parasitising the gastrointestinal tracts of goats and sheep in East Pakistan. *Pakistan J. Vet. Sc.* 2: 54 – 62.
- Islam KS, Ahmed S (1987). Effect of temperature, light and darkness on the hatching of eggs and development of L3 larvae of gastrointestinal helminths and migratory behavior of larvae in grass blades. *Indian J. Parasitol.* 11: 45 – 49.
- Islam MK, Begum N, Alim MA, Mondal MMH (2005). A simple method for harvesting the third-stage infective larvae of trichostrongylid nematodes of ruminants and their implications in the screening of pesticides. *Bangladesh Vet. J.* 39: 49 – 58.
- Lloyd (1983). Effect of pregnancy and lactation of infection. *Vet. immunol. and immunopathol.* 4: 153 – 176.
- Minato K, Kimura E, Shintoku Y, Uga S (2008). Effect of temperature on the development of free-living stages of *Strongyloides ratti*. *Parasitol. Res.* 102: 315 – 319.
- Patel MD, Nauriyal DS, Hasnani JJ, Gupta RS (2001). Prevalence of gastrointestinal parasitism in goats maintained under semi intensive and field management systems. *Indian J. Vet. Med.* 21: 99 – 101.
- Paul HS (1965). *In vitro* Cultivation Procedures for Parasitic Helminths. *Advances in Parasitology Academic Press. London*, 3: pp. 159 – 218.
- Rahman MH (1969). Taxonomic study of the helminth parasites encountered in the abomasums of ruminants in East Pakistan. M. Sc. Thesis, Department of Parasitology, Bangladesh Agricultural University, Mymensingh.
- Rahman MH, Mondal MMH, Ahmed S (1996). Introduction to helminth parasites of Animals and birds in Bangladesh. First Edition. Mr. Taehsins Mostafa, Pvt. Ltd.
- Rehbein S, Visser M, Winter R (2002). Helminths of goats in Germany. *Berliner und Munchener Tierarztliche Wochenschrift*. 113: 427 – 431.
- Rossanigo CE, Gruner L (1994). Relative effect of temperature and moisture on the development of strongyle eggs to infective larvae in bovine pats in Argentina. *Vet. Parasitol.* 55 (4): 317 – 325.
- Samad MA (2008). *Animal Husbandry and Veterinary Science*. LEP Publication.No. 11, BAU campus, Mymensingh, Bangladesh. pp. 244 – 245.
- Sommerville RI, Murphy CR (1983). Reversal of order of ecdysis in *Haemonchus contortus* (Nematoda). *J. Parasitol.* 69 (2): 368 – 371.
- Soulsby EJJ (1982). *Helminth, Arthropod and Protozoa of Domesticated Animals*. 7thedn., Bailliere Tindall, London, p.809.
- Steel RJ, Torrie DS (1980). *Statistics of Veterinary and Animal Science*, 1st Edition, Blackwell Science Ltd., USA, pp. 41 – 49.
- Stringfellow F (1986). Cultivation of *Haemonchus contortus* (Nematoda: Trichostrongylidae) from infective larvae to the adult male and the egg-laying female. *J. Parasitol.* 72 (2): 339 – 345.
- Tripathi JC (1977). Effect of different temperatures, on the eggs of gastrointestinal nematodes of goats under laboratory conditions. *Indian J. Anim. Sci.* 47(11): 739 – 742
- Tripathi JC (1980). Effect of different temperatures, on the eggs of gastrointestinal nematodes of goats under controlled conditions. *Int. Vet. J.* 57: 719 – 723
- Veglia F (1916). The anatomy and life history of *Haemonchus contortus*. The Third and Fourth Reports of the Director of Veterinary Research. Union of South Africa, pp. 349 – 500.